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(72) Inventors: HARRIS, Jeffrey, D. ; 15 Flatstone, The Woodlands, TX 77381 (US). HSU, Kuang, T. ; 71 N. Misty Morning Trace, The Woodlands, TX 77381 (US). PODOLSKI, Joseph, S. ; 3 Pebble Hollow Court, The Woodlands, Tx 77381 (US).			

(54) Title: MATERIALS AND METHODS FOR IMMUNOCONTRACEPTION

(57) Abstract

A method for specifically inducing transient infertility or permanent sterility in a host animal by selective vaccination with specific zona pellucida proteins or immunocontraceptively active fragments thereof. Novel zona pellucida DNA sequences encoding specific zona pellucida proteins are disclosed.

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- 1 -

TITLE: MATERIALS AND METHODS FOR  
IMMUNOCONTRACEPTION

5

**CROSS REFERENCE TO RELATED APPLICATION**

This application is a continuation-in-part of U.S. Application Serial No. 08/012,990, filed January 29, 1993, which is a continuation-in-part of U.S. Application Serial No. 07/973,341, filed on November 9, 1992.

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**FIELD OF THE INVENTION**

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This invention relates generally to the production and use of zona pellucida proteins, and more particularly to novel DNA sequences encoding zona pellucida proteins, to recombinant materials and methods for producing such proteins and to materials and methods for selectively effecting either transient infertility or permanent sterility in mammals through use of naturally occurring and recombinant zona pellucida proteins.

20

**BACKGROUND OF THE INVENTION**

The present invention relates to a method for inducing reproducible transient infertility or sterility in a mammal by inducing in that mammal antibodies directed to proteins found in the zona pellucida of that mammal's oocytes. The invention also relates to purified, isolated DNA sequences encoding the zona pellucida proteins herein designated "ZPA" and "ZPB" and "ZPC" from various mammalian species. The invention is further directed to pharmaceutical compositions capable of inducing antibody production in a subject mammal.

- 2 -

The zona pellucida (ZP) is a complex matrix surrounding the mammalian oocyte, formed of glycoproteins secreted by ovarian cells. Zona pellucida glycoproteins perform a variety of functions. For example, the mouse ZP proteins previously designated ZP2 and ZP3 are complexed into long filaments which are cross-linked by the protein designated ZP1 in the ZP matrix providing structural integrity to the matrix. Wassarman, P.M., *Annu. Rev. Biochem.* 57:415-442 (1988). In addition to its structural role, mouse ZP3 has been shown to be a sperm receptor in the ZP matrix. Bleil, J.P. and Wassarman, P.M., *Cell* 20: 873-882 (1980). Following binding of sperm to ZP3 and the subsequent induction of the sperm acrosome reaction on the surface of the ZP, ZP2 acts as a secondary sperm receptor that is necessary for the maintenance of sperm binding to the egg. Bleil *et al.*, *Dev. Biol.* 128: 376-385 (1988). Because of its role in the maintenance of the oocyte and in sperm-oocyte interactions, the ZP represents a logical target for design of contraceptive agents which interfere with the fertilization process.

Various groups have undertaken an immunological approach in attempts to interfere with ZP functions and thus to decrease fertility in immunized animals. See, Dunbar *et al.* In: *International Congress on Reproductive Immunology*. T. Wegman and T. Gills (eds.). London: Oxford Press, pp. 505-528 (1983); and Dunbar *et al.* In: *Mechanisms and Control of Animal Fertilization*. J. Hartman (ed.) Academic Press, New York, pp. 139-166 (1983). These studies showed that active immunization of mammals with ovarian homogenates decreased fertility. However, the large number of components in such homogenates made the identification of antigens responsible for the decrease in fertility nearly impossible. In addition, the use of such a complex mixture creates a potential for unwanted and potentially harmful side-effects.

Research by various investigators using chromatographic methods including SDS polyacrylamide gel electrophoresis (PAGE) and high pressure liquid chromatography (HPLC) have resulted in the identification of

- 3 -

numerous zona pellucida proteins from a variety of mammalian species. Data compiled by Timmons and Dunbar in "Perspectives in Immunoreproduction: Conception and Contraception"; pp. 242-260, Mathur, S. and Fredericks, C.M. eds.; New York, Hemisphere Publishing Co (1988), as described below, illustrate examples of zona pellucida proteins that have been characterized.

Zona pellucida proteins isolated from pig include: PZI, a 40-110 kD protein isolated by Dunbar *et al.*, *Biol. Reprod.* 24:1111 (1981); PZII, a 70-110 kD protein, PZIII, a 95-118 kD protein, and PZIV, an 18-25 kD protein, all isolated by Dunbar *et al.*, *Biol. Reprod.* 32:619 (1985); 90K, a 89-119 kD protein, 65K, a 61-83 kD protein, 55K, a 47-66 kD protein, and 25K, an 18-26 kD protein, all isolated by Hedrick, J.L. and Wardrip, N.J. *Biochem.* 157: 63 (1986); ZP1, an 82-118 kD protein, ZP2, a 58-96 kD protein, ZP3 (PPZA), a 40-74 kD protein, and ZP4, a 21 kD protein, all isolated by Subramanian *et al.*, *Biol. Reprod.* 24:933 (1981); 87K (ZP1/ZP2), a 77-97 kD protein, 58K, a 40-70 kD protein both isolated by Yurewicz *et al.*, *Biol. Reprod.* 29: 511 (1983); deglycosylated PZI, a 35 kD protein; PZII, a 55 kD protein; and PZIII, an 80 kD protein all isolated by Skinner and Dunbar as described in *Immunological Approaches to Contraception and the Promotion of Fertility*, G. P. Talwar (ed.) New York: Plenum pp. 251-268 (1986); and deglycosylated ZP3 having a molecular weight of 45 kD isolated by Sacco *et al.*, *J. Reprod. Fertil.* 76:575 (1986).

Isolated rabbit zona pellucida proteins include: RZI, RZII, and RZIII, having molecular weights of 68-125 kD, 80-100.5 kD, and 100-132 kD respectively, all isolated by Dunbar *et al.*, *Biol. Reprod.* 24:1111 (1986); 25 ZP1, ZP2, and ZP3 having molecular weights of 100-118 kD, 83-110 kD, and 80-92 kD respectively, all isolated by Sacco *et al.*, *Proc. Soc. Exp. Biol. Med.* 167:318 (1981); deglycosylated RZI, and RZII having molecular weights of 65 kD, and 80kD respectively, both isolated by Skinner and Dunbar and described in *Immunological Approaches to Contraception and Promotion of Fertility*. G.P. Talwar (ed.). New York: Plenum, pp. 251-268 (1986); and

- 4 -

deglycosylated RZIII, a 90 kD protein isolated by Timmons and Dunbar, *Biol. Reprod.* **36:** 1275 (1987).

A number of mouse zona pellucida proteins have been isolated including: ZP1, ZP2, and ZP3 having molecular weights of 200 kD, 120 kD, 5 and 83 kD respectively, all isolated by Bleil and Wassarman *Dev. Biol.* **76:**185 (1980); and ZP1 and ZP2 having molecular weights of 166-122 kD and 90-92 kD respectively, isolated by Sacco *et al.*, *Proc. Soc. Exp. Biol. Med.* **167:** 318 (1981). The differences in the molecular weights of mouse ZP1 and ZP2 as reported by Bleil *et al.* and Sacco *et al.* may be due to the 10 fact that Bleil used 2D-PAGE under non-reducing conditions while Sacco used 2D-PAGE under reducing conditions.

The cat zona pellucida proteins CZI and CZII were isolated by Maresh and Dunbar *J. Exp. Zool.* **244:**299 (1987) and have molecular weights of 50-110 kD and 90-110 kD respectively.

15 Maresh and Dunbar *J. Exp. Zool.* **244:**299 (1987), have also isolated the dog zona pellucida proteins DZI, DZII, and DZIII which have molecular weights of 50-110 kD, 70-95 kD, and 90-100 kD respectively.

Sacco *et al.*, *Proc. Soc. Exp. Biol. Med.* **167:**318 (1981) described squirrel monkey ZP1, ZP2, ZP3, and ZP4 having molecular weights 20 of 63-78 kD, 63-70 kD, 47-51 kD, and 43-47 kD respectively. In the same publication

Sacco *et al.* described human ZP1, ZP2, and ZP3 having molecular weights of 80-120 kD, 73 kD, and 59-65 kD respectively.

To date, few mammalian zona pellucida genes or proteins have 25 been isolated and sequenced. None has been successfully used to produce an effective immunocontraceptive. A lack of consensus among those of skill in the art regarding the number and characteristics (e.g. molecular weight) of proteins present in the zona pellucida of various mammalian species, and difficulties in purifying these heavily glycosylated proteins have hampered

- 5 -

attempts to utilize zona pellucida proteins to produce an effective immunocontraceptive with predictable function.

A number of groups have had success in cloning cDNAs or genes encoding various mammalian zona pellucida proteins.

5                   Ringuette *et al.*, *Dev. Biol.*, 127:287-295 (1988) and Liang *et al.*, *Mol. Cell. Biol.*, 10:1507-1515 (1990), reported cloning of mouse DNA encoding zona pellucida proteins ZP3 and ZP2, respectively. The clones were obtained by screening mouse cDNA libraries with anti-ZP3 and anti-ZP2 antibodies. No sequence homology was found between mouse ZP3 and ZP2.

10                  Ringuette *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:4341-4345 (1986), reported isolation of a partial cDNA clone for mouse ZP3, which clone hybridized with total genomic DNA of mouse, rat, dog, cow, and human, but not with pig or rabbit genomic DNA unless the hybridization was performed at very low stringency. The full length ZP3 cDNA characterized 15 by Ringuette *Dev. Biol.* 127:287-295(1988) represents a germ-line specific mRNA having relatively short 5' and 3' untranslated regions and an open reading frame of about 1317 nucleotides with an additional 200-300 nucleotide poly-A tail. Ringuette also found that rat, rabbit, dog, and cow ovary transcribes mRNA which hybridized to the mouse ZP3 cDNA and that the 20 ZP3 transcripts had similar molecular weights. Liang *et al. Mol. Cell. Biol.*, 10:1507-1515 (1990), showed that the nucleic acid and deduced amino acid sequence of ZP2 is distinctly different from that of ZP3 although it had the same short motif of 5' and 3' untranslated regions. The ZP2 mRNA is reported to have single open reading frame of 2,139 nucleotides which codes 25 for a polypeptide of 80,217 Daltons representing 713 amino acids.

Chamberlin and Dean, *Dev. Biol.* 131:207-214 (1989) and Kinloch, R.A. *et al.*, *Proc. Nat. Acad. Sci. USA*, 85:6409-6413 (1988) have reported the cloning of the mouse ZP3 gene. The mouse ZP3 gene is reported to have 8 exons and 7 introns in a transcription unit of 8.6 kbp.

- 6 -

Kinloch *et al.*, *Dev. Biol.* 142:414-421 (1990), reported cloning of hamster genomic ZP3 DNA from a hamster genomic DNA library screened with mouse ZP3 DNA as a probe. The hamster ZP3 gene has a transcription unit of 7900 nucleotides and was found to contain 7 introns and 8 exons. The 5 hamster ZP3 protein is approximately 81% homologous to mouse ZP3 protein. The hamster transcript contained 1266 nucleotides, six less than mouse ZP3 mRNA.

Chamberlain and Dean, *Proc. Natl. Acad. Sci. USA* 87:6014-6018 (1990), reported the cloning of human ZP3 from a human 10 genomic DNA library using mouse ZP3 cDNA as a probe. The human ZP3 gene is composed of 8 exons in a transcription unit of 18.3 kbp. The exons are almost identical in size to the eight exons of mouse ZP3 and the nucleotide sequence of the coding region is 74% homologous. The human ZP3 transcript is very similar to mouse ZP3 mRNA. Both have short 5' and 3' untranslated 15 regions, and both have a single open reading frame of 1272 nucleotides that encodes a 424-amino acid protein.

U.S. Patent No. 4,996,297, to Dunbar, reported the isolation of three rabbit zona pellucida clones encoding rabbit ZP1 and ZP2 proteins, using anti-ZP1 and anti-ZP2 antibodies as screening probes. The sequences 20 designated as P2 and P3 in Figure 4 of the Dunbar patent represent rabbit ZP cDNAs of 812 and 1705 nucleotides respectively.

Schwoebel *et al.*, *J. Biol. Chem.* 266:7214-7219 (1991), isolated and characterized a full length cDNA (designated rc 55) encoding the 25 55-kD rabbit zona pellucida protein using cross-species affinity purified antisera. The protein encoded by this cDNA has some similarity to the mouse ZP2 protein described by Liang. However, comparisons of rc 55 with the mouse ZP3 protein revealed no homology.

The functional activities of the cloned ZP DNAs and their 30 encoded proteins have not been fully characterized and neither has their potential use as immunocontraceptives been demonstrated.

- 7 -

In order to develop a useful zona pellucida product for use in fertility control, particularly in the form of a vaccine, it is highly desirable to purify, isolate, and characterize zona pellucida proteins from a species of an animal of interest. Because of factors such as the purity of such proteins needed for vaccine production, and the high cost and numerous problems associated with purification of these proteins, it would be highly desirable to ascertain the DNA and amino acid sequences of zona pellucida proteins of a specific species of interest. Having such known, isolated and characterized zona pellucida proteins, the function of each zona pellucida protein may be understood and a fertility control product may be designed based upon the specific functional characteristics of a particular zona pellucida protein and for a particular mammalian species.

It would be thus highly useful and desirable to provide isolated, purified, sequenced, and characterized recombinant zona pellucida proteins which would permit the development of fertility control products possessing specific reproducible effects in eliciting transient and/or permanent infertility. Such products, where used to elicit transient infertility, would desirably have long lasting effects so as to minimize the number of times the immunocontraceptive agent must be administered to maintain infertility.

20

#### SUMMARY OF THE INVENTION

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The present invention provides novel methods and materials for inducing either reproducible transient or permanent infertility effects in female mammals, including humans, by selective administration of homologous and/or heterologous mammalian species ZP proteins or immunocontraceptively active fragments thereof hereinafter designated as ZPA, ZPB and ZPC. By "reproducible" is meant that, unlike prior art attempts to induce transient infertility by administration of ZP proteins (in the form of mixtures of such proteins), this invention achieves its transient infertility effects by the administration of ZPA and/or ZPB in a form such that the duration of

- 8 -

transient infertility is controllable and can be maintained in an on or off condition in a controllable and/or predictable fashion. This is achieved primarily through administration of the highly pure ZPA and ZPB proteins or immunocontraceptively active fragments thereof of this invention, e.g., in recombinant form and thus essentially devoid of ZPC. By immunocontraceptively active fragments is meant a ZP protein fragment capable of inducing infertility.

In one of its aspects, the present invention provides methods for inducing reproducible transient infertility in a mammal by administering to a subject female mammal a zona pellucida protein (or fragment thereof) selected from the group consisting of mammalian ZPA, and ZPB, and combinations thereof in doses effective to stimulate production in said mammal of antibodies which recognize ZPA or ZPB proteins of said mammal. It is presently preferred that mammalian ZPA and ZPB for use in such methods be derived from the same mammalian species as the subject mammal although the use of heterologous species proteins is also contemplated. Use of purified isolates of mammalian ZPA or ZPB protein such as obtained by chromatographic separatory procedures is contemplated. Use of proteins produced by recombinant methods is expected to be most preferred.

According to another aspect of the invention, methods are provided for inducing permanent sterility in a female mammal by administering to a subject female mammal a recombinant mammalian ZPC protein (or fragment thereof) in a form essentially devoid of ZPA and/or ZPB, in a dose effective to stimulate production in said female mammal of antibodies which recognize the ZPC protein of said mammal. As is the case with induction of transient infertility, use of homologous species ZPC is preferred, but not required, and the protein may be derived from natural sources or produced by recombinant methods. Modified ZPC proteins including but not limited to palmitoylated and chitosan modified proteins are also contemplated by the present invention.

- 9 -

Presently preferred ZPA, ZPB, and ZPC proteins for veterinary application of the transient infertility and sterility inducing methods include porcine, rabbit, canine, feline, bovine, and cynomolgus monkey ZP proteins.

In another of its aspects, the present invention provides  
5 pharmaceutical compositions for use in inducing reproducible transient infertility in a female mammal (including humans) comprising an effective dose of a zona pellucida protein (or fragment thereof) selected from the group consisting of mammalian ZPA, and ZPB (substantially free of ZPC), in combination with one or more pharmaceutically acceptable carriers, diluents  
10 and adjuvants. Modified ZPA and ZPB proteins (for example, palmitylated or chitosan modified) are also contemplated by the present invention.

According to another aspect of the present invention, novel purified and isolated DNA sequences are provided which encode porcine ZPA, ZPB, and ZPC, as illustrated by the DNA sequences set out in SEQ ID NOS. 1, 3, and 5. Also, provided are purified and isolated DNA sequences encoding: rabbit ZPC, as illustrated by the DNA sequence set out in SEQ ID NO. 7; canine ZPA and ZPC, as illustrated by the DNA sequences set out in SEQ ID NOS. 9 and 11; feline ZPA, ZPB, and ZPC, as illustrated by the DNA sequences set out in SEQ ID NOS. 13, 15, and 17; bovine ZPA, ZPB,  
20 and ZPC, as illustrated by the DNA sequences set out in SEQ ID NOS. 19, 21, and 23; human ZPA and ZPB as illustrated by sequences set out in SEQ ID NO. 42 and 40, respectively, and as contained as human DNA inserts in lambda phage clones A1 and A4, (ZPA) and as contained in human DNA inserts in lambda phage clones 1-1 and 4-9 (ZPB).

25 Polynucleotide sequences of the invention are useful for the production of ZPA, ZPB and ZPC proteins by recombinant methods and as probes for the isolation of heterologous species polynucleotides encoding corresponding zona pellucida proteins by hybridization methods.

Also provided by the present invention are novel host cells,  
30 especially unicellular eucaryotic and prokaryotic cells, stably transformed or

- 10 -

transfected with polynucleotides of the invention in a manner allowing expression of the ZP proteins (or immunologically significant fragments thereof) in the host cells. Host cells expressing such ZP products, when grown in a suitable culture medium, and particularly useful for large scale production processes wherein the desired polypeptide products, in glycosylated or non-glycosylated form are isolated from the cells or the medium in which the cells are grown.

Recombinant polypeptides provided by the invention thus comprise ZPA, ZPB and ZPC, and full equivalents of such zona pellucida proteins including both glycosylated and non-glycosylated forms, variants and immunologically active fragments thereof which retain substantial biological activity, i.e., at least one of the biological activities of the zona pellucida protein discussed herein, e.g., the ability to stimulate the production of antibodies as discussed herein upon administration to a mammal. Such immunologically active fragments may be defined as containing at least one epitope effective to stimulate the production of antibodies upon administration to a mammal in accordance with this invention.

In another aspect of the invention, a method is provided for the isolation of nucleic acid sequences encoding other mammalian ZPA, ZPB, and ZPC proteins by hybridization under stringent conditions of heterologous species ZPA, ZPB, and/or ZPC probes to cDNA or genomic DNA libraries, derived from the mammalian species of interest.

More particularly, it is an aspect of the invention to provide a method for the isolation of nucleic acid sequences encoding human ZPA and ZPB by hybridization under stringent conditions of sequences encoding ZPA and/or ZPB from heterologous species.

Other aspects and advantages of the present invention will be readily understood upon consideration of the following detailed description of presently preferred embodiments thereof, reference being made to the figures wherein:

- 11 -

#### DESCRIPTION OF THE FIGURES

Fig. 1 is a diagrammatic representation of the plasmid vector

5 pZ90;

Fig. 2 is a diagrammatic representation of the plasmid vector

5 pZ98; and

Fig. 3 is a diagrammatic representation of the plasmid vector

5 pZ156.

Fig. 4 is a diagrammatic representation of the alignment of the  
Eco R1 fragments encoding human ZPB.

10 Fig. 5 is a diagrammatic representation of the plasmid vector  
pZ169.

Fig. 6 is a diagrammatic representation of the plasmid vector  
5 pZ145.

#### DETAILED DESCRIPTION OF THE INVENTION

15 The present invention is directed to mammalian zona pellucida proteins characterized in three major classes: ZPA, ZPB, and ZPC. This classification scheme has resulted from repetitive screening of various mammalian ovarian cDNA libraries and retrieval of clones which encode proteins showing significant homology in three distinct groups, designated 20 herein as ZPA, ZPB and ZPC. Although similarity is seen between DNA sequences encoding ZPA, ZPB, or ZPC between animal species, very little homology is found between the individual species' ZPA, ZPB, and ZPC proteins.

25 DNA sequences encoding zona pellucida proteins A, B, and C and their deduced amino acid sequences for various mammalian species ZPs are presented in SEQ ID NOS. 1-24. It is understood that the DNA sequence of a particular animal may vary slightly due to the phenomenon of allelic variation. Small differences in the precise DNA sequence between animals or slight errors due to the inefficiency of sequencing procedures are to be

- 12 -

expected. Such variants are included within the scope of the present invention.

The zona pellucida DNA sequences described above were obtained from ovarian cDNA libraries screened with specific zona pellucida antibodies or known zona pellucida DNA probes. Comparison of isolated sequences to published protein or DNA sequences and with other clones as they were isolated was used to classify and identify the clones as described above.

The term "zona pellucida protein" is meant to include full length proteins ZPA, ZPB, and ZPC, as well as expected variants, immunologically active fragments or peptides contained within these proteins. The term "zona pellucida DNA" is meant to include those nucleic acid sequences encoding zona pellucida protein or fragments thereof.

The three major classes of mammalian zona pellucida proteins have been determined on the basis of homology within the DNAs encoding ZP proteins of a variety of mammalian species. ZPA includes those peptides previously, variously described in the literature as ZP1, ZP2, and ZP4; ZPB includes those peptides previously, variously described as ZP3 $\alpha$  and rc 55; and ZPC includes those peptides previously variously described as ZP3 $\beta$  and ZP3.

The homology of various species of zona pellucida proteins within a specific class as compared with a consensus sequence for each class is shown in Table 1. The consensus sequence was derived using the Microgenie® Sequence Analysis Program (Beckman Instruments, Inc. Spinco Division, Palo Alto, CA). The minimum percent of aligned sequences which must have the same residue at a given position for that residue to be included in the consensus sequence was 50%. The DNA sequences corresponding to the amino acid consensus sequences for ZPA, ZPB, and ZPC proteins are set out in SEQ ID NOS 25, 26, and 27, respectively.

- 13 -

TABLE 1  
HOMOLOGY OF DEDUCED ZP PROTEINS AMINO ACIDS

		ZPA	ZPB	ZPC
	DOG	78.9%	--	77.3%
5	CAT	78.4%	70.9%	77.5%
	COW	77. 2%	80.4%	77.2%
	PIG	73.0%	77.8%	79.0%
	RABBIT	70.1%	74.6%	71.3%
	MOUSE	61.6%	--	69.6%
10	HUMAN	--	--	76.9%
	HAMSTER	--	--	70.5%

The deduced amino acid sequences of the various species of zona pellucida proteins suggest approximate unglycosylated molecular weights of 75 kD, 55 kD, and 45 kD for ZPA, ZPB, and ZPC, respectively. A more 15 detailed analysis of both DNA sequence homology and deduced amino acid sequence homology is set out as Examples 13, 14, and 15.

It has surprisingly been found that administration of a specific class of zona pellucida protein to a host animal results in a specific immunocontraceptive effect and that selection of the appropriate ZP protein 20 for administration allows induction of desired contraceptive results, in terms of permanent sterility or transient infertility. For example, vaccination of an animal with zona pellucida protein C induces antibody titers in that animal which recognize endogenous ZPC resulting in loss of oocytes from the animal's ovary, thereby causing permanent sterility. In contrast, vaccination 25 of an animal with zona pellucida protein A, B or combinations thereof induces antibody titers which do not recognize ZPC, but recognize ZPA and/or ZPB. This results in cycling, infertile animals for the time period during which

- 14 -

anti-ZPA and/or anti-ZPB antibody titers remain high. When such antibody titers fall, the infertility effect is diminished, and the animal regains fertility.

Vaccination with the purified, isolated, and characterized ZPA, ZPB, or ZPC proteins is seen to exert a specific effect on the immunized

5      animal if an autoimmune response is triggered wherein the autoantibodies generated specifically recognize the immunized animals' own specific zona pellucida protein. This self-recognition for antibodies induced according to the present invention may be defined and characterized by the ability of serum antibodies to recognize at least one epitope present on a homologous species  
10     zona pellucida protein.

In the preferred method of the invention, an animal is immunized with a recombinant ZPA, ZPB, or ZPC or fragments thereof. The recombinant protein or peptide may be of homologous species or derived from a heterologous species zona pellucida which shares common epitopic determinants, with the proviso that such common epitopic determinants function to induce the desired autoimmune response.

The recombinant protein or peptide fragment may be chemically conjugated to immune enhancing agents such as Keyhole Limpet Hemocyanin (KLH), and Muramyl dipeptide (MDP), and the like, or alternatively may be  
20     provided in the form of a fusion protein, e.g., with foreign protein amino acids at the amino and/or carboxy terminus. Fully conventional methods for stimulating the production of antibodies upon administration of the proteins or fragments of this invention are well known; similarly, passive immunization techniques involving administration of antibodies *per se*, e.g., anti-ZPA  
25     antibodies, anti-ZPB antibodies, or anti-ZPC antibodies, to the zona pellucida proteins or fragments of this invention is also within the scope of the invention. For details, see Dean, PCT Application WO90/15624 whose disclosure is entirely incorporated by reference herein.

Thus, to induce permanent sterility in a dog, recombinant  
30     canine ZPC may be employed which is expressed as a bacterial fusion protein

- 15 -

(or conjugated to immune enhancing agents) wherein active canine ZPC protein is conserved and available for interaction with antigen presenting cells.

The expressed protein is then administered to a host dog and induces an autoimmune response in which generated antibodies recognize canine zona

5 pellucida protein C. This autoimmune effect, which specifically recognizes dog ZPC protein or its aggregates, induces permanent sterility in the vaccinated dog, which sterility is associated with a loss of oocytes from the dog's ovary.

Alternately, a non-homologous species ZPC, such as  
10 recombinant porcine ZPC or peptides thereof which are cross-reactive with canine ZPC, can be administered to a dog to achieve similar sterilizing effects. The sterilizing effect, however, is only realized when antibodies capable of recognizing the host's own native zona pellucida are induced (or administered in the context of passive immunization).

15 In an alternative embodiment of the present invention, the administration of a host species' own A and/or B class zona pellucida protein, or a related A and/or B protein from another species which induce antibodies against the host's ZPA and/or ZPB proteins results in an infertility effect which is distinct from that produced by ZPC class antigens. The

20 physiological effect of vaccination with the ZPA and ZPB proteins is a transient one. "Transient infertility" is herein defined as infertility which is maintained when antibodies against self-zona pellucida proteins are sustained in the host animal's circulation at a contraceptively effective concentration (e.g., at titers of approximately 1:250 in the dog) and which infertility is

25 diminished when antibodies against self fall below a contraceptively effective lower limit. The reduction in antibodies against self-zona pellucida results in restoration of fertility without evidence of major physiological changes in the ovary. Typically, the reduction in antibody titers occur by natural processes in the mammalian host, but other methods of reducing antibody titers are

30 within the scope of the invention.

- 16 -

Contraceptively effective antibody titers against self zona pellucida proteins A and B required to maintain infertility will vary with the species of vaccinated animal as well as with the species of recombinant ZPA or ZPB peptide administered, but may readily be determined, for example, by

5 testing a panel of the desired animal species with varying doses of the specific antigen, measuring the induced titer of anti-self antibodies by known ELISA techniques, and correlating the titers with reproductive indicators, e.g., cycling, hormone levels, and the like. In general, antibody titers greater than 1:250 are contraceptively effective.

10 Based on amino acid sequence homologies, it is expected that all zona pellucida proteins of a particular class contain functional epitopes which are cross-reactive between mammalian species. However, absent characterization and identification of such functional cross-reactive epitopes, a preferred, selective contraceptive agent is a homologous species zona pellucida protein or antibody thereto.

15 The present invention will be more completely understood upon consideration of the following illustrative examples of the practice thereof wherein: Example 1 addresses the isolation of DNAs encoding porcine species ZPA, ZPB and ZPC; Example 2 relates to isolation of rabbit ZPC DNA; Example 3 relates to isolation of DNAs encoding canine ZPA and ZPC; Example 4 addresses isolation of feline DNAs encoding ZPA, ZPB and ZPC; Example 5 relates to cloning and isolation of DNAs encoding bovine species ZPA, ZPB and ZPC; Examples 6 and 7 describe immunocontraceptive treatment of dogs with naturally-derived porcine zona pellucida proteins; 20 Example 8 relates to serochemical studies on animals treated in Examples 6 and 7; and Examples 9 and 10 address recombinant production of a canine ZPC fusion protein and its immunocontraceptive use in dogs. Example 11 relates to the isolation of DNAs encoding human ZPA and ZPB by methods described herein. Example 12 relates to the isolation and sequencing of DNAs 25 encoding cynomolgus monkey ZPA, ZPB and ZPC. Examples 13-15 relate 30

- 17 -

to the comparison of the DNA sequence and the deduced amino acid sequence of mammalian ZPA, ZPB, and ZPC, respectively. Example 16 relates to the immunization of cynomolgus monkey using HSPZ and fractionated HZPC. Example 17 relates to the mapping of mammalian zona pellucida protein epitopes. Example 18 describes the immunization of dogs using recombinant ZPC proteins. Example 19 relates to the vaccination of cows and cats with recombinant ZP proteins.

### Example 1

## Isolation of DNA Sequences Encoding

## 10 Porcine Zona Pellucida Proteins ZPA, ZPB, and ZPC.

A cDNA library in  $\lambda$ gt11 was commercially prepared by Clonetech, Palo Alto, CA, from an ovary isolated from a 14 week old pig and was screened using an anti-ZP3 $\beta$  antibody obtained from E.C. Yurewicz and described in Keenan *et al.*, *Biol. Reprod.*, 44:150-156 (1991). Eight candidate clones were identified.

A degenerate DNA oligonucleotide probe (19bps) was constructed to represent all possible sequences of a short portion of the N-terminus porcine ZP3 $\beta$  as described in Yurewicz *et al.*, *J. Biol. Chem.*, 262:564-571, (1987). The degenerate probe sequence is set out in SEQ ID NO. 28.

Southern analysis of the eight candidate clones isolated by expression screening with the degenerate DNA oligonucleotide probe resulted in hybridization with two of the eight candidates. The two clones recognized by the degenerate probe were then subcloned into the pBS KS plasmid (STRATAGENE Cloning Systems, La Jolla, CA) for sequence analysis using the sequence enzyme and the protocol described in the SEQUENASE® Manual (U.S. Biochemical, Cleveland, OH). One of the clones, B-8, having an insert size of approximately 1200 base pairs, included a sequence homologous to the

- 18 -

N-terminal sequence of mouse ZP3, previously identified by Ringuette *et al.*, *Dev. Biol.*, 127:287-295, (1988). The remaining clone, B-6, had an insert size of approximately 1000 base pairs. Neither hybridizing clone contained the C-terminal portion of the gene, as suggested by the lack of homology to the  
5 mouse ZP3 gene in this region.

The 14-week porcine ovarian library was then rescreened by DNA hybridization. Approximately 150,000 PFUs were plated on agar plates with *E. coli* Y1090. After overnight incubation at 37°C, nylon membrane lifts of plaques were prepared and screened using the B6 and B8  
10 clones derived above isolated by screening with the degenerate oligonucleotide probe set out in SEQ ID NO. 28.

Filters were prehybridized in a solution containing 5X saline, sodium phosphate, EDTA buffer (SSPE), 5X Denhardt's Reagent, 100µg/ml salmon sperm DNA, 30% formamide and 0.5% SDS for three hours at 42°C.  
15 Approximately 50 ml of the prehybridization solution was used for 12 filters (132 mm). After prehybridization, 10 ng of freshly radiolabeled DNA probe in 30% formamide, 5X SSPE was added. The probes were heat denatured at 95°C for 3-5 minutes and hybridization with the DNA probes continued overnight at 42°C. The hybridized filters were then washed twice with 100 ml  
20 of 5X SSPE at 55°C, for approximately one hour each wash. The filters were then rinsed with 250 ml of 5X SSPE at room temperature and allowed to air dry. The dried filters were exposed to x-ray film at -70°C using intensifier screens for at least eight hours and the films were developed for visual analysis.

25 Among the additional clones isolated were two clones including the C-terminal portion of the porcine ZP3 $\beta$  gene. One clone,  $\lambda$ 5-1, was subcloned into plasmid pBS KS and sequenced. This plasmid, termed pZ57, contained a ZP DNA insert having 1266 base pairs and appeared to encode the full length amino acid sequence of porcine ZP3 $\beta$  as compared with known  
30 mouse ZP3. Alignment of the deduced amino acid sequence of the clone with

- 19 -

the known N-terminal amino acid sequence of ZP3 $\beta$  reported by Yurewicz *et al.*, *J. Biol. Chem.*, 262:564-571 (1987), and an internal peptide sequence of ZP3 $\beta$  corresponding to amino acids 255-274 as provided by E.C. Yurewicz confirmed the identity of this clone as encoding porcine ZP3 $\beta$ .

5       The DNA sequence of this clone, termed porcine ZPC, is set out in SEQ ID NO. 5 and its deduced amino acid sequence is set out in SEQ ID NO. 6.

10      The 14-week porcine ovarian cDNA library was further screened using rabbit zona pellucida rc 55 cDNA as a probe [described in Schwoebel *et al.*, *J. Biol. Chem.*, 266:7214-7219, (1991)].

15      One candidate clone of approximately 1700 base pairs,  $\lambda$ 2-1, was isolated and was transferred into the sequencing plasmid pBS KS. The DNA sequence and deduced amino acid sequence of the porcine DNA insert was determined using the method described in the SEQUENASE® manual (US Biochemical Corporation, Cleveland, Ohio). The sequenced clone contained 1620 base pairs and included a full length copy of the porcine ZP3 $\alpha$  gene as confirmed by alignment of the deduced amino acid sequence with portions of the known protein sequence of porcine ZP3 $\alpha$  provided by E.C. Yurewicz between amino acids 206-222, 271-279, and 328-344. The DNA sequence 20 of this clone, termed porcine ZPB, is set out in SEQ ID NO. 3. Its deduced amino acid set out in SEQ ID NO. 4.

25      The 14-week porcine ovarian library was further screened using the procedure described above and using a DNA probe encoding canine ZPA protein (as obtained in Example 3 below, SEQ ID NO. 9). A single clone,  $\lambda$ 3-5 having approximately 1300 base pairs, was obtained representing the N-terminal 60% of the theoretical porcine ZPA gene as estimated by the size of the clone in relation to the ZP2 gene isolated from mouse by Liang *et al.*, *Mol. Cell. Biol.* 10:1507-1515 (1990), and rabbit by Dunbar, U.S. Patent No. 4,996,297, and dog (see Example 3 below).

- 20 -

This clone was then used to rescreen the porcine ovarian library. Three additional clones were obtained, two small clones and one clone large enough to contain the full length sequence. The large candidate clone,  $\lambda$ B, having approximately 2200 base pairs, was sequenced, and the data showed this ZPA clone to lack only approximately seven base pairs of the full length sequence including the ATG start codon when aligned with the mouse ZP2 gene and the canine ZPA gene described in Example 3. The DNA sequence of this clone, termed porcine ZPA, is set out in SEQ ID NO. 1. Its deduced amino acid sequence is set out in SEQ ID NO. 2.

This isolated porcine clone included sequences corresponding to published sequences of three identified porcine zona pellucida proteins, ZP1 (80kD), ZP2 (62kD) as disclosed in U.S. Patent No. 4,996,297 to Dunbar and ZP4 (21kD) as disclosed by Hasegawa *et al.*, Abst. No. 382, *Meeting Soc. Study Reprod.* July, 1991. These results suggest that a singular clone encodes one zona pellucida protein which previously had been thought to exist as three separate proteins, i.e., ZP1, ZP2, and ZP4. This further suggests that only three major porcine zona pellucida genes encode three major zona pellucida proteins which here are termed ZPA, ZPB, and ZPC. ZPA includes those proteins previously identified as ZP1, ZP2, and ZP4. ZPB corresponds to ZP3 $\alpha$  and ZPC corresponds to previously identified ZP3 $\beta$ . Yurewicz *et al.* *J. Biol. Chem.*, 262:564-571, (1987).

**Example 2**  
**Isolation and Purification of DNA Sequences**  
**Encoding Rabbit ZPC Protein**

Ovaries were removed from five week old rabbits and mRNA was prepared using the Fast Track™ mRNA isolation kit in accordance with the procedure described in the *Fast Track™* instruction manual, version 3.1, catalog No. K1593-02 (Invitrogen, San Diego, CA). A Lambda Librarian™

- 21 -

kit (Invitrogen, San Diego, CA) was used to prepare cDNA and to clone cDNAs into λgt10 according to the manufacturer's instructions. Approximately 150,000 PFUs were plated on agar plates with *E. coli* Y1090. After overnight incubation at 37°C, nylon membrane lifts of colonies were 5 prepared and screened with a porcine ZPC DNA probe using the screening procedures described for Example 1. The probe used was the porcine ZPC sequence as set out in SEQ ID NO. 5.

Two positive clones, λR4 and λR5, hybridized with the porcine ZPC DNA. The size of each of these clones as estimated in agarose gels was 10 approximately 1300 base pairs. Both λR4 and λR5 were sequenced as described for Example 1. The sequences were identical except that λR5 contained four additional nucleotides at the 5' end. The determined DNA sequence was approximately 75% homologous to the DNA sequence encoding porcine ZPC.

15 The DNA sequence encoding rabbit ZPC protein is set out in SEQ ID NO. 7. Its deduced amino acid sequence is set out in SEQ ID NO. 8.

Rabbit ZPA and ZPB proteins have been previously identified by Dunbar in U.S. Patent No. 4,996,297 as P2 and P3, respectively.

20

### Example 3

#### Isolation of DNA Sequences Encoding Canine Zona

#### Pellucida Proteins ZPA and ZPC

A 16 week canine ovarian cDNA expression library was commercially prepared by Clone Tech, Palo Alto, CA, in λgt11 generally 25 following the methods described in Example 1. The canine ovarian cDNA library was screened using antibodies raised against heat solubilized canine zona pellucida. Heat solubilized canine zona pellucida (HSDZ) was prepared generally following the procedures described in Dunbar *et al. Biochemistry*,

- 22 -

19:356-365, (1980) except ganged razor blades were used to mince the ovaries.

Rabbits were immunized with 250 µg HSDZ and 250 µg MDP. Two additional boosts followed at approximately three week intervals. The 5 resultant rabbit serum was used to screen the canine ovarian cDNA expression library. Seven candidate clones were obtained. Cross-hybridization experiments were performed by Southern blot analysis as follows. The largest clone, λ26-1, having approximately 1300 base pairs, was first used as a probe against all of the other clones in Southern blots. Three other clones were 10 identified. The largest of the remaining clones, λ20-1 and λ7-1, having approximately 800 and 1000 base pairs respectively, were then used as probes in Southern blots. These probes identified no additional clones. This cross hybridization analysis of the seven candidate clones to each other indicated that four of these clones were related, e.g. four clones hybridized to λ26-1 15 while the remaining three λ20-1, λ7-1, and λ19-3 were independent.

The largest of the four related clones, λ26-1, was subcloned into pBS KS plasmid for sequence analysis according to the procedure described in Example 1. The analyzed sequence demonstrated the presence of a long open reading frame of 1278 base pairs encoding a protein of 20 approximately 426 amino acids. Comparison of the deduced amino acid sequence of this clone with the sequences of known zona pellucida proteins, indicated this clone encoded a protein related to mouse ZP3 (ZPC) as reported by Ringuette *et al.*, *Dev. Biol.* 127:287-295 (1988), hamster ZP3 as reported by Kinloch *et al.*, *Dev. Biol.*, 142:414-421 (1990), human ZP3 as reported by 25 Chamberlin *et al.*, *Proc. Natl. Acad. Sci. USA* 87:6014- 6018 (1990) and porcine ZPC protein (see Example 1). The DNA sequence of this clone, termed canine ZPC, is set out in SEQ ID NO. 11. Its deduced amino acid sequence is set out in SEQ ID NO. 12.

30 The remaining three independent candidate clones were subcloned into the pBS KS plasmid for sequence analysis as described above.

- 23 -

The determined sequence of the 800 base pair clone, λ20-1, was compared with known ZP sequences by computer analysis as described above and was found to be related to the mouse ZP2 (ZPA) [Liang *et al.*, *Mol. Cell. Biol.* 10:1507-1515 (1990)] and porcine ZPA (see Example 1).

5       The 800 base pair fragment from λ20-1, was then used as a hybridization probe to rescreen the canine cDNA library. Two additional candidate clones were identified, the larger of which, λ7A, having approximately 2800 base pairs, was subcloned into pBS KS plasmid for sequence analysis. Comparison of this sequence with known sequences  
10      encoding zona pellucida proteins suggested the candidate clone λ7A contained a full length ZPA sequence, but an incorrect N-terminal sequence, e.g., the clone contained an additional 600 base pairs as determined by alignment with known mouse ZP2 and rabbit ZPA sequences referenced in Example 1. The second candidate clone, λ9-2, having approximately 1000 base pairs, was then  
15      subcloned into the plasmid pBS KS and sequenced. The sequence of the second clone indicated the presence of a correct N-terminal sequence, but included only approximately the N-terminal 40% of the full length clone as determined by alignment with the mouse ZP2 and rabbit ZPA genes. Overlap  
20      of the two cDNA clones, however, provided the full length sequence.

20       The appropriate pieces of each clone were subcloned as follows to generate the correct full length zona pellucida clone containing a 2028 base pair open reading frame encoding a protein of approximately 676 amino acids. The λ7A DNA was digested with Eco RI to yield two insert fragments (2000 bps and 800 bps). These two fragments were each subcloned into pBS KS  
25      yielding pZ36 and pZ37, respectively. Plasmid pZ37 carried the C-terminal portion of this sequence. The λ9-2 DNA insert was removed from the λ vector and subcloned into pBS KS to yield pZ38. Plasmid pZ36 was digested with Hind III to remove approximately 1350 bps of the N-terminal portion of the λ7A gene fragment (about 850 bps of nonsense DNA and 500 bps of coding sequence). This digestion also removed one of the Eco RI insert ends  
30

- 24 -

and left a single Eco RI site. The pZ37 Eco RI insert was then moved into the single remaining Eco RI site in the modified pZ36 (pZ36 ΔI) to reestablish the relative DNA structure orientation that existed in the λ7A insert (1450/2800 bps). This combined plasmid was then opened with Hind III and 5 the Hind III fragment from pZ38 carrying the N-terminal ZP DNA sequence was inserted to create plasmid pZ39 which is a pBS KS carrying the full length canine ZPA sequence. The DNA sequence of this canine ZPA gene is set out in SEQ ID NO. 9. Its deduced amino acid sequence set out in SEQ ID NO. 10.

10

#### Example 4

##### Isolation of DNA Sequences Encoding Feline Zona Pellucida Proteins ZPA, ZPB, and ZPC

Ovaries were isolated from five cats approximately three to four months in age. Messenger RNA was isolated from six ovaries using the Fast 15 Track™ mRNA Isolation Kit (Invitrogen, San Diego, CA, Catalog No. K1593-02) using the protocol provided with the kit. cDNA was prepared using the protocol and cloned into λgt10 as described in Example 2.

Approximately 150,000 plaque forming units (PFUs) were plated on agar plates with *E. coli* Y1090. After overnight incubation at 37°C, 20 nylon transfer membranes were used to prepare and screen plaque lifts. Plaques were screened using a mixture of DNA probes in equal proportions encoding porcine ZPA, ZPB, and ZPC proteins and using the hybridization procedure as described for Example 2. A total of 81 positive clones were identified. Twelve of these clones were plaque-purified. Southern analysis 25 of these clones using porcine ZPA, ZPB, and ZPC DNAs individually as probes indicated that seven of these clones encoded ZPC proteins and one clone encoded a ZPA protein. Four of the clones contained inserts which could not be separated by Eco RI digestion

- 25 -

Five of the ZPC clones were between 1200-1350 base pairs in length. One clone,  $\lambda$ C-112, having approximately 1350 base pairs was subjected to sequence analysis as described above and its deduced amino acid sequence was found to be approximately 70% homologous to the canine ZPC protein obtained in Example 3. The DNA sequence of this feline ZPC clone is set out in SEQ ID NO. 17. Its deduced amino acid sequence is set out in SEQ ID NO. 18.

The single feline ZPA clone,  $\lambda$ C-116, was sequenced and found to be approximately 2215 base pairs in length. The deduced amino acid sequence was approximately 75% homologous to the canine ZPA protein characterized in Example 5. The DNA sequence of this feline ZPA clone is set out in SEQ ID NO. 13. Its deduced amino acid sequence is set out in SEQ ID NO. 14.

The remaining 69 positive clones were rescreened using porcine ZPB DNA as a probe (SEQ ID NO. 3). Ten positive clones were obtained. The largest clone,  $\lambda$ C-1, contained approximately 1.7 kilobases as determined by agarose gel electrophoresis. This clone was sequenced, and its deduced amino acid sequence was found to be approximately 80% homologous to the porcine ZPB protein described in Example 1. The DNA sequence of this feline ZPB clone is set out in SEQ ID NO. 15. Its deduced amino acid sequence is set out in SEQ ID NO. 16.

#### Example 5

##### Isolation of DNA Sequences Encoding Bovine Zona Pellucida-Proteins ZPA, ZPB, and ZPC

A cDNA library was constructed from a five month bovine ovary by the method described in Example 2. The bovine ovarian library was screened with DNA hybridization probes representing each of the classes of zona pellucida proteins using a mixture of equal proportions of porcine

- 26 -

DNA probes encoding ZPA (SEQ ID NO. 1), ZPB (SEQ ID NO. 3), and ZPC (SEQ ID NO. 5) proteins, as described for Example 2 and using the procedures described for Example 1. Initial screening yielded three candidate clones. Southern analysis of these clones with individual porcine ZPA, ZPB,  
5 and ZPC DNA probes used in the initial screening indicated that one of the clones,  $\lambda$ B2, having approximately 650 base pairs, encoded ZPA. A second clone,  $\lambda$ B-1 having approximately 1000 base pairs encoded ZPB. A third clone,  $\lambda$ B14, having approximately 1200 base pairs, encoded ZPC.

10 The bovine ovarian library was then rescreened with the mixed porcine ZP DNA probes. Two additional clones were obtained and identified by Southern analysis as encoding ZPC.

15 The Eco RI inserts of the ZPA, ZPB, and largest ZPC clone were subcloned and their DNA sequences analyzed. The sequences encoding these bovine ZPA, ZPB and ZPC fragments were set out in SEQ ID NOS. 19, 21, and 23, respectively. Their deduced amino acid sequences are set out in SEQ ID NOS. 20, 22, and 24, respectively.

#### Example 6

##### Immunization of Dogs with Heat-Solubilized Fractionated Porcine Zona Pellucida

20 Heat-solubilized, porcine zona pellucida (HSPZ) was prepared generally following the procedures described by Dunbar *et al. Biochemistry*, 19:356-365, (1980) but using a hand powered meat grinder instead of the Zonamatic described. Following isolation, the zona pellucida protein was solubilized in 0.1 M sodium carbonate buffer, pH 9.6, and was dialyzed  
25 extensively against 6M urea. The resultant solution, a volume of 2-3ml containing approximately 12 $\mu$ g of HSPZ, was subjected to isoelectric-focusing in a BIORAD Rotofor isoelectric-focusing chamber as follows. An isoelectric gradient was established using 1% ampholytes having a pI range of 3-10. The

- 27 -

zona pellucida protein was introduced into the mid-range chamber (pI 7.0) and allowed to focus for approximately four hours at 4°C or until the voltage stabilized.

Twenty isoelectrically focused fractions were collected and analyzed by SDS PAGE and Western blot analysis for pig zona pellucida proteins. Acidic fractions having a pI range of approximately 3.5-5.5 and which contained the porcine zona pellucida proteins were combined. The fractions were dialyzed into 0.1M carbonate buffer, pH 9.6 and concentrated to approximately 3mg/ml. This antigenic preparation was used to vaccinate animals as described below. Analysis of this antigenic preparation by two-dimensional gel electrophoresis indicated the presence of ZPA and ZPB protein. However, ZPC was not revealed to be present in this preparation.

The HSPZ antigenic preparation was added to a 50/50 water oil emulsion with incomplete Freund's adjuvant (Sigma, St. Louis, MO) containing 250 $\mu$ g of MDP per dose. One ml of the 50/50 water oil emulsion contained 0.425 ml paraffin oil, 0.075 ml mannide monooleate, and 0.5 ml PBS containing 250  $\mu$ g threonyl-MDP (SYNTEX Corporation) and the amount of HSPZ described in Table 3 below.

Four random breed dogs aged 10-12 weeks were immunized with HSPZ using the regimen described in Table 2.

TABLE 2

		<u>mg HSPZ</u>
	Prime	0
	Boost #1	1.0
25	Boost #2	0.25
	Boost #3	0.2
	Boost #4	1.0
	Boost #5	1.0

- 28 -

The antisera produced by these animals was monitored via ELISA methodology. By week 17 antibody titers against self, e.g. against canine zona pellucida proteins, had reached a maximum (8-16K by ELISA) and thereafter began to drop.

5 At week 36, one animal was unilaterally ovariectomized and the removed ovary was sectioned and stained with periodic acid schiff stain (PAS) for histological examination. The ovary appeared normal, as evidenced by the presence of follicles in all stages of development. At week 52, two of the  
10 four test animals were observed to exhibit estrus behavior. The remaining two test animals exhibited estrus behavior at approximately one and a half years when the first two test animals experienced their second heat. All test animals were bred repeatedly with competent males and by artificial insemination, however, none became pregnant. During this same period, animals in various test regimens in which no self titers were obtained, as  
15 described in Example 10, became pregnant when presented with the same males or artificial insemination techniques.

Two weeks following the breeding sessions, e.g. at 54 weeks, the two early cycling animals were unilaterally ovariectomized and the removed ovaries were sectioned for histological examination. The ovaries  
20 appeared normal for this stage of follicular activity despite the functional infertility demonstrated.

**Example 7**  
**Vaccination With Porcine ZPC Protein**

A purified porcine ZPC protein (ZP3 $\beta$ ) was obtained from E.  
25 Yurewicz, prepared as described in *J. Biol. Chem.*, 262:564-571, (1987).

Vaccines were prepared by adding 167 $\mu$ g purified porcine ZPC protein (ZP3 $\beta$ ) to a 50/50 water-oil emulsion with complete Freund's adjuvant (Sigma No. F5881, St. Louis MO), for the priming dose or with Incomplete

- 29 -

Freund's Adjuvant (Sigma No. F5506, St. Louis, MO) containing MDP as described in Example 6 for the booster doses.

Five random breed dogs of approximately 10-12 weeks of age were injected with the ZPC vaccine preparation described above using the 5 regimen described in Table 3.

TABLE 3

			<u>mg of ZPC</u>
10	Prime	Time 0	0.167
	Boost	Week 3	0.167
	Boost	Week 6	0.167
	Boost	Week 28	0.167

Each animal's antibody titer versus self- zona proteins, e.g., versus canine zona pellucida proteins, was monitored by ELISA, using the method described in Dunbar, *Two Dimensional Gel Electrophoresis and Immunological Techniques*, 1987. ELISA microtiter plates were coated with HSDZ in antigen-coating buffer (0.1M sodium carbonate, pH 9.6). Biotinylated rabbit-antidog IgG was used as the second antibody. ABC reagent (Avidin-biotinylated peroxidase complex) and O-phenylene diamine dihydrochloride with a peroxide substrate was used for visualization. Only 15 two animals produced antibodies versus self achieving peak self-antibody titers of 16K by week 4. The other three animals produced no self-antibody titers but achieved peak antibody titers of 4K against porcine zona pellucida protein. During the period of time between week 20 and week 36, all dogs were observed to exhibit estrous behavior. The animals were bred repeatedly with 20 proven males. Only the two animals having antibody titers versus self zona pellucida proteins remained infertile. All other animals in the study became pregnant.

- 30 -

Two weeks after estrous and breeding the two infertile dogs exhibiting self-antibody titers were unilaterally ovariectomized and the removed ovaries were sectioned and stained with PAS for histological examination. The histological examination revealed abnormal morphology in the ovaries of the infertile dogs. No evidence of ongoing folliculogenesis was seen and the ovaries were depicted of oocyte-containing follicles. In addition, no primordial oocytes were seen.

#### **Example 8**

In an attempt to better understand the immune response and different physiological effects obtained in the two studies described in Examples 6 and 7, antisera produced in each test group was analyzed by Western Analysis against a variety of antigens including natural porcine ZPC, heat-solubilized dog zona pellucida (HSDZ), recombinant dog ZPA and ZPC, and recombinant pig ZPC. Western blots were probed with antiserum obtained from the test animals of Example 6, e.g., animals immunized with isoelectric focused, heat-solubilized porcine zona pellucida, and with antiserum obtained from the two test animals of Example 7 which contained antibodies against self-zona proteins.

The data demonstrate no recognition of recombinant porcine or canine ZPC by antisera from infertile, but cycling dogs immunized with heat solubilized porcine zona pellucida which contained no demonstrable ZPC by PAGE analysis, however, natural ZPC, HSDZ and recombinant canine ZPA were recognized. In contrast, antisera obtained from infertile dogs whose ovaries were depleted of oocytes recognized recombinant ZPC protein, i.e., the polypeptide backbone.

- 31 -

A key difference in the antibody recognition of antigen was that only the antisera obtained from dogs having ovaries devoid of oocytes appeared to recognize the recombinant dog ZPC antigen. Infertile dogs whose antisera strongly recognized natural ZPC, HSDZ, and recombinant dog ZPA 5 demonstrated no recognition of recombinant dog ZPC.

Given that autoimmunity is essential for a contraceptive effect, these data suggest that infertility without histologically evident ovarian dysfunction can be obtained in dogs via an autoimmune response against dog ZPA antigens. In contrast, histologically confirmed ovarian dysfunction, i.e., 10 loss of oocytes, which would result in permanent sterility, requires the generation of antibodies which specifically recognize homologous species ZPC protein.

#### Example 9 Expression of Recombinant ZP Proteins

##### 15 1. Construction of Expression Vectors

The plasmid vector pZ90 shown in Fig. 1 was constructed from fragments of the plasmids pUC9 (Vierstra & Messing, *Gene* 19:259-268 (1982)) and p $\beta$ gal2 (Queen, *J. Mol. App. Gen.* 2:1-10 (1983)). The single *Pvu* II restriction site present in p $\beta$ gal2 was converted to a *Sal* I site using a *Sal* I polylinker adaptor purchased from New England Biolabs. The DNA sequences between the new *Sal* I site and a pre-existing *Sal* I site were excised by digestion with *Sal* I, religated and screened for the reduced size plasmid. 20

A *Cla* I - *Nde* I fragment of the modified p $\beta$ gal2 plasmid which carried the  $\lambda$ C1 repressor gene, the  $\lambda$ pR promoter and the Lac Z gene ( $\beta$ -galactosidase) was inserted into pUC9 between its *Acc* I and *Nde* I restriction sites. The pUC9 plasmid carries the ampicillin resistance (Amp $R$ ) gene and col E1 replication origin (ori) needed to maintain the plasmid in *E. coli* cells. The combination plasmid was further modified to convert the *Bam* 25

- 32 -

HI site 3' of the ATG initiation codon (ATG GAT CCN) to a Bgl II site 5' of the ATG initiation codon (AGATCTATG). This was accomplished by partially digesting the plasmid with Rsa I. One of the several digestion points was about 20 bps 5' of the Bam HI restriction site. When the partially digested plasmid was digested with Bam HI, some of the plasmids produced were nearly full length. A synthetic oligomer (GTACTAAGGAAGATCTATGGATCC) (SEQ ID NO. 29) was produced to replace the sequence that had been removed (GTACTAAGGAGGTTGTATGGATCC) (SEQ ID NO. 30). The net effect of this replacement was the substitution of 3 bps to create the Bgl II restriction site. A DNA fragment containing approximately 3000 base pairs of the Lac Z gene was then excised by restriction digestion with Bgl I and Ban II and was followed by insertion of a synthetic oligomer containing a Bam HI site. The plasmid was cut with Bgl I and Ban II, and then treated with nuclease S1 to create blunt ends. A Bam HI linker (New England Biolabs) was inserted at the blunt ends of the digested plasmid. Next a Pvu II restriction site between the λCI repressor gene and the ori sequence was converted to a Hind III site using a synthetic linker. The Pvu II restriction site was cut with Pvu II, and a Hind III linker (New England Biolabs) was ligated to the blunted ends. Because the remaining lac Z sequence was missing the first 8 codons of the natural sequence, these 8 codons were replaced by synthesizing a synthetic oligomer that began with a Bgl II site and encoded the lac Z wild type gene product ( $\beta$ gal) N-terminal sequence.

The synthetic oligomer was prepared by synthesizing four oligomers having the sequences set out in SEQ ID NO. 31 (oligomer 1), SEQ ID NO. 32 (oligomer 2), SEQ ID NO. 33 (oligomer 3), and SEQ ID NO. 34 (Oligomer 4). Oligomers 2 and 3 were phosphorylated by treating with kinase and ATP to add phosphate to the 5' end. Oligomers 1 and 2 were then hybridized to oligomers 3 and 4, respectively, by incubation at 100°C followed by a slow cooling in 200 $\mu$ M NaCl. The resultant oligomer had the sequence

- 33 -

set out in SEQ ID NO. 35. The synthetic oligomer as set out in SEQ ID NO. 35 had Bgl II-Pvu II ends and was substituted for the Bgl II-Pvu II sequence of the plasmid by restriction digestion of the plasmid and ligation with the oligomer.

5           The resultant plasmid was termed pZ90 and is shown in Figure 1. The plasmid pZ90 can be used to express recombinant proteins by heat induction, using the heat labile λCI repressor. The heat-inducible repressor and promoter of pZ90 was next replaced with the chemically inducible promoter ptac (Amann *et al.*, *Gene* 25:167-178 (1983)). The ptac promoter  
10          is controlled by the lac repressor, a product of the lac I gene (Farabaugh, *Nature* 279:765-769 (1978)). The Lac I gene was obtained from pMC9 (Miller *et al.*, *The EMBO Journal* 3:3117-3121 (1984)) by use of PCR methodology as described by Innis and Gelfand, In: *PCR Protocols: A Guide to Methods and Applications*, Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. (eds.), pgs 1-12, Academic Press, Inc., San Diego, CA. The  
15          primers used were complimentary to the Lac I promoter at one end and the Lac I gene termination codon at the opposite end. The N-terminal primer carried a Hind III site and the C-terminal primer carried a tac promoter sequence followed by a Bgl II site. The N-terminal primer had the sequence  
20          set out in SEQ ID NO. 36. The C-terminal primer had the sequence as set out in SEQ ID NO. 37 which includes a Dra 3 site having the sequence 5'-CACAAATGTG-3'. The resulting lac I - ptac DNA fragment having Hind III and Bgl II restriction sites at its respective ends was then used to replace the Hind III - Bgl II fragment of pZ90 which carried the λCI repressor and λPrR  
25          promotor. This replacement yielded the plasmid pZ98 shown in Fig. 2.

## II. Insertion of Recombinant ZP DNA

DNA sequences encoding porcine ZPC were prepared by the PCR procedures described above (Innis & Gelfand) from the plasmid pZ57 prepared in Example 1, which contains the full length porcine ZPC sequence

- 34 -

obtained from λgt11 clone 5-1 described for Example 1. During the PCR procedure the porcine ZPC gene was modified by using primers that did not include the leader sequence and the hydrophobic tail. The N-terminal primer used had the sequence set out in SEQ ID NO. 38 which included an internal

5      Bam HI restriction site having the sequence 5'-GGATCC-3'. The C-terminal primer used had the sequence as set in SEQ ID NO. 39 includes a Sal I restriction site having the sequence 5'-CTCGAG-3' and an internal Xho I restriction site having the sequence 5'-CTCGAG-3'. The modified ZPC gene contained base pairs 105 to 1154 encoding ZPC amino acids 1-350.

10     To the 5' end of the modified porcine ZPC gene was added a Bam HI restriction site, and to the 3' end was added an Xho I site, a Hexa-CAT-codon sequence (CAT)<sub>6</sub>, a termination codon, and a Sal I restriction site. This modified porcine ZPC gene was inserted into the Bam HI - Sal I restriction site of pZ98 to yield the porcine ZPC expression vector, 15    plasmid pZ156 shown in Fig. 3. The (CAT)<sub>6</sub> sequence produces a C-terminal hexahistidine (His<sub>6</sub>) amino acid sequence in the recombinant fusion protein which permits purification of the fusion protein by immobilized metal in affinity chromatography.

20     In a similar manner as described above, the plasmid pZ156 when digested with Bam HI and Xho I, may be used to receive any other recombinant ZP gene or gene fragment for expression as a βgal fusion protein which can be purified by metal ion affinity chromatography.

### III. Expression of Porcine ZPC Fusion Protein in *E. coli*

The expression vector pZ156 (Fig. 3) was transformed into *E. coli* strain Top 10F' (Invitrogen, San Diego, CA) by the procedure of Chung *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2172-2175 (1989). The transformed *E. coli* cell line was termed Strain ZI 156, and was used to express recombinant porcine ZPC-βgal fusion protein.

- 35 -

Bacterial cultures of ZI 156 were grown in Luria Broth (LB) containing 100  $\mu$ g/ml ampicillin at 30°C until the cell density reached an OD<sup>600</sup> of approximately 1.5. Isopropyl beta-D-thiogalactopyranoside (IPTG) (3ml of 100mM solution/ 1 media) was added to induce expression from the tac promoter, and the cells were further incubated at 30°C for 2-3 hours. The cells were harvested by centrifugation, and the resulting cell pellet was frozen at -70°C.

The frozen cell pellets were suspended in 10 mM EDTA (1g/2-2.5 ml) and twice sonicated at 50% power for 3 minutes, cooling in an ice bath between each sonication. The cell lysate was then centrifuged at 3300 x g for one hour and the hard pellet was retained. This lysis procedure was repeated using the hard pellets.

In order to remove residual EDTA, the final hard cellular pellet was dispersed in a small volume of water by a brief burst of sonication, the suspension was centrifuged, and the supernatant discarded. The washed pellet was thoroughly resuspended in Buffer A, (6M guanidine hydrochloride (GuHCl), 100 mM Na H<sub>2</sub>PO<sub>4</sub>, 10 mM TRIS pH 8, at approximately 0.5 ml per original gram of cell pellet). The suspension was centrifuged at 10,000 x g for 45 seconds and the supernatant was retained while the pellet was discarded.

The retained supernatant was loaded onto a Ni column (in Buffer A) and the column was washed with 10 column volumes of Buffer A. The column was next washed with 5 volumes each Buffers B-D, each containing 8M urea, 100mM NaH<sub>2</sub>PO<sub>4</sub>, and 10 mM TRIS, and having successively reduced pH values of 8, 6.3, 5.9 for Buffers B, C, and D, respectively. The recombinant pZPC- $\beta$ gal fusion protein eluted with Buffer E, at pH 4.5 as shown by screening by Western Blot analysis using rabbit anti-HSDZ and anti-HSPZ as probes. Further elution may be accomplished using Buffer F (pH 2.5) (8M GuHCl, 200 mM Acetic Acid).

- 36 -

The fusion protein obtained by this protocol was prepared in its final dose for injection into a host animal by adjusting the final volume to 0.5 ml in 8M urea, and adding it to 0.5 ml adjuvant as described above. Each dose was injected subcutaneously into a test animal.

5

#### Example 10

##### Vaccination of Dogs with Recombinant

##### ZPC- $\beta$ gal Fusion Protein

Eleven mixed breed dogs approximately 5-6 months of age were randomly selected from test animals previously treated at approximately 2  
10 months of age with heat solubilized porcine zona pellucida or chromatographically purified porcine ZP3 $\beta$  in combination with various biopolymers as adjuvants and drug releasing vehicles. Six weeks post first injection, i.e., three and a half months of age, all test animals had achieved antibody titers versus HSPZ in the range of 2-16K as determined by ELISA.  
15 However, none of the test animals achieved antibody titers against self-antigen, e. g., HSDZ.

At 5-6 months of age, five of the test animals were then injected with a loading dose of the porcine ZPC- $\beta$  gal fusion protein prepared as described for Example 9. The recombinant ZPC- $\beta$  gal fusion protein  
20 produced in Example 9 was adjusted to the desired dose in a final volume of 0.5ml 8M urea and combined with 0.5 ml adjuvant. The adjuvant, N-acetyl-D-glucosaminyl- $\beta$ (1,4)-N-acetyl muramyl-L-alanyl-D-isoglutamine (GMDP), 250 $\mu$ g, was dispersed in 0.42 ml mineral oil, 0.157 ml L-121 block polymers, and 0.02 ml Tween 80. Each dose was injected subcutaneously  
25 into the five test animals. The remaining 6 animals were maintained as controls.

- 37 -

Following a total of four injections given at 2-3 week intervals, antibody titers versus self antigen, e.g., HSDZ, were obtained in all test animals, with peaks in the range of 2-8 K as measured by ELISA.

Some of the control animals began to cycle beginning at approximately 9 months of age, and by 11 months of age, 4 of 6 control animals had experienced their first estrus. In contrast, none of the 5 test animals which had received recombinant ZPC- $\beta$  gal fusion protein had cycled during this same time period. However, although the first estrus was delayed for several months in the test animals, they eventually began to cycle. Two of the five vaccinated dogs became pregnant during their second estrus after immunization while a third dog became pregnant during its third estrus after immunization; however, the two remaining test animals remain infertile through three estrus cycles and nearly two years after vaccination.

#### Example 11

#### Isolation of Human DNA Sequences Encoding Human Zona Pellucida Proteins ZPA and ZPB

A human genomic DNA library purchased from Stratagene (catalog no. 946203) was used for the isolation of DNA sequences encoding human ZP proteins. The library consisted of 9-23 kb inserts of human DNA (from placenta tissue of a male caucasian) cloned into the Lambda Fix<sup>TM</sup>II vector (Stratagene). Approximately 40,000 pfus were plated on *E. coli* strain LE 392 (Stratagene, catalog no. 200266), as described in the Stratagene protocol, but replacing MgSO<sub>4</sub> with MgCl<sub>2</sub>. After overnight incubation, nylon membrane lifts of the plaques were prepared and screened with <sup>32</sup>P-labelled porcine ZPA cDNA (SEQ ID NO. 1) and with <sup>32</sup>P-labelled porcine ZPB cDNA (SEQ ID NO. 3) as described in Example 2.

Three clones 1-1, 2-2, and 4-9 were shown to hybridize to the porcine ZPB cDNA (SEQ ID NO. 3). Clones 1-1 and 4-9 were deposited

- 38 -

with the American Type Culture Collection, (ATCC) 12301 Parklawn Drive, Rockville, Maryland, on January 27, 1993 under ATCC Accession Nos. 75406 and 75405, respectively. Human DNA inserts were isolated from these clones and analyzed by restriction endonuclease digestion with Eco RI and 5 Southern blot analysis as described in Example 1. Table 4 shows the results of Eco RI digestion of these clones.

Table 4  
HUMAN GENOMIC ZPB EcoRI INSERTS

CLONES			
10	Fragment	1-1	2-2
	A		2.8 kb
	B	2.2 kb	
	C	2.0 kb	
15	D	1.5 kb	
	E	0.2 kb	
	F	3.2 kb	3.2 kb
	G	0.7 kb	

Southern blot analysis revealed four Eco RI fragments which were judged to carry ZPB coding sequences based on hybridization to the 20 porcine ZPB cDNA (SEQ ID NO. 3). Clone 1-1 DNA included a 2.2 kb, 2.0 kb, and 1.5 kb Eco RI fragments which so hybridized. Clone 2-2 DNA included a 2.8 kb Eco RI hybridizing fragment. Clone 4-9 DNA included a 2.8 kb and a 1.5 kb Eco RI fragment which hybridized to the porcine ZPB cDNA probe. All inserts additionally included a 3.2 kb non-hybridizing Eco 25 RI fragment; inserts from clones 1-1 and 4-9 both provided 0.2 kb non-hybridizing fragments; and clone 1-1 additionally provided a 0.7 kb non-hybridizing fragment.

- 39 -

Further restriction analysis revealed the fragment alignment shown in Figure 4. Six of the fragments (A-F) were subcloned into pBSKS for sequence analysis, as described in Example 1. Preliminary sequence analysis confirmed the fragment alignment shown in Figure 4, and suggested  
5 that the complete coding sequence of the human ZPB gene may be from clones 1-1 and 4-9. This was confirmed by nucleotide sequence analysis of the inserts, and comparison of the sequences with the feline ZPB sequence (SEQ ID NO. 15) and porcine ZPB sequence (SEQ ID NO. 3). The DNA sequence and deduced amino acid sequences for human ZPB are set out as  
10 SEQ ID NO. 40 and 41, respectively.

Clones hybridizing to the porcine ZPA cDNA (SEQ ID NO. 1) under the conditions described in Example 1 were also isolated. Two positive clones, A1 and A4 were identified. The clones were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville,  
15 Maryland 20852, on January 27, 1993 under ATCC Accession Nos. 75404 and 75403 respectively. Southern blot analysis revealed that these clones contain all or part of the human ZPA gene. DNA was isolated from these clones and was analyzed by Bgl II, Hind III, and Not I restriction endonuclease digestion and Southern blot analysis as described in Example 1.  
20 The size of the A1 clone DNA insert is approximately 11.6 kb, and that of the A4 clone is approximately 13.2 kb. Two of the Bgl II fragments which hybridized with the porcine ZPA cDNA (SEQ ID NO 1) were subcloned into pBSKS for sequence analysis, as described in Example 1. Sequence analysis revealed that A1 and A4 collectively contain the human ZPA gene as  
25 supported by comparison to sequences with the porcine ZPA cDNA (SEQ ID NO. 1) and the canine ZPA cDNA (SEQ ID NO. 11). The complete DNA sequence and the deduced amino acid sequence are set out as SEQ ID NOS. 42 and 43, respectively.

- 40 -

### Example 12

#### Isolation and Sequencing of DNA Encoding Cynomolgus Monkey ZPA, ZPB, and ZPC

Cynomolgus monkey cDNA libraries were constructed in λgt10 5 as described below. Briefly, a set of ovaries were collected from two female cynomolgus monkeys aged 1.5 years and 2 years, and a second set from three females aged 3 years, 4 years, and 14 years of age. Messenger RNA was isolated using the Fast Track™ mRNA isolation kit following the manufacturer's instructions. The cDNA was prepared using the Lambda 10 Librarian™ (Invitrogen, as described in Example 2) kit following the protocol provided with the kit. The cDNA was packaged into lambda phage heads 15 using the Protoclone® (Promega, Madison, WI) λgt10 EcoRI arms plus the Packagene® (Promega) lambda DNA packaging system following the manufacturer's instructions. This procedure generally produced libraries with a titer of greater than 1 x 10<sup>6</sup> pfu/ml. The monkey cDNA library was then screened using porcine ZPA, ZPB, and ZPC probes isolated from the porcine cDNA as described in Example 1. Screening was accomplished by preparing 20 duplicate plaque lifts using Nytran® nylon filters (0.2μM pore size). The filters were prehybridized in a solution of 5x SSPE (43.83 g/l of NaCl, 6.9 g/l of NaH<sub>2</sub>PO<sub>4</sub>, H<sub>2</sub>O, 1.85 g/l of EDTA, pH 7.4), 5x Denhardts Reagent (1 g/l of Ficoll [type 400], 1 g/l of polyvinylpyrrolidone and 1 g/l bovine serum albumin), 100μg/ml sonicated, denatured salmon sperm testes DNA, 30% formamide, and 0.5% SDS, for 3 hrs. at 42°C. Radio-labelled probes were prepared using [ $\alpha$  - <sup>32</sup>P] -dATP and the Prime-a-Gene® (Promega) labelling 25 system. After prehybridization, 10 ng of freshly radio-labelled probe was heat denatured at 95°C for 5 minutes in 50% formamide and 100 μg/ml sonicated, denatured salmon testes DNA, and was added to the filters. The hybridization was carried out at 42°C for 15-24 hours. The hybridized filters were then washed twice with 100 ml of 5X SSPE at 55°C, for approximately one hour

- 41 -

each wash. The filters were then rinsed in 250 ml of 5X SSPE at 55°C and allowed to air dry. The dried filters were exposed to x-ray film (Kodak XAR5, Eastman Kodak, Rochester NY ) at -70°C using two intensifying screens (Kodak X-OMATIC™) for at least eight hours. The film was then developed for visual analysis.

Exhaustive screening of the two cynomolgus monkey ovarian cDNA libraries using all of the porcine probes yielded a total of 12 candidate clones. Southern hybridization revealed that only one of these clones ( $\lambda$  CM 4-2) hybridized to the porcine ZPA probe. This clone contained an insert of 560 bp. Sequencing of the insert was performed using the Sequenase® Version 2 kit (U.S. Biochemicals, Cleveland, Ohio) according to the manufacturer's instructions. Sequencing revealed that the 560 bp insert was homologous to the 3' end of other mammalian ZPA genes. The 560 bp fragment represents just under 25% bp of the full-length sequence and contains an open reading frame of 492 bp which would encode a protein of 164 amino acids. The DNA sequence and the deduced amino acid sequence of the cynomolgus monkey ZPA cDNA is set out as SEQ ID NOS. 44 and 45, respectively.

Exhaustive screening of the cynomolgus monkey ovarian cDNA libraries with the porcine ZPB probe yielded a single ZPB candidate clone having an insert of 866 bp. Sequence analysis suggests that the insert includes the C-terminal 50% of the expected full-length sequence. The DNA sequence and deduced amino acid sequence of the monkey ZPB insert are set out as SEQ ID NOS. 46 and 47, respectively. Screening of monkey ovarian cDNA libraries with the porcine ZPC DNA probe yielded only partial ZPC clones, the largest ( $\lambda$  CM1-1) having an insert of approximately 1300 bp which contains just over 50% of the C-terminal portion of the full-length sequence based on comparison to known ZPC clones, (particularly the human ZPC clone). The clone contains an open reading frame of 672 bp which would encode a protein of 224 amino acids. The clone also contains stop codons

- 42 -

immediately 5' to the coding sequence in all three reading frames. The DNA sequence and the deduced amino acid sequence of the cynomolgus monkey ZPC clones are set out as sequence ID NOS 48 and 49 respectively.

**Example 13**

**5 Comparison of ZPA DNA and Deduced Amino Acid Sequences**

Table 5 shows a comparison of the DNA and deduced amino acid sequence of mammalian ZPAs.

TABLE 5  
ZPA HOMOLOGY

## PROTEIN HOMOLOGY

	Mouse	Rabbit	Pig	Cow	Dog	Cat	Monkey	Human
Mouse	--	61.0%	54.2%	60.8%	57.9%	56.9%	57.2%	58.9%
Rabbit	73.0%	--	63.0%	69.8%	66.2%	64.6%	65.1%	68.9%
Pig	69.0%	75.6%	--	79.9%	69.6%	70.2%	56.9%	63.9%
Cow	70.5%	79.0%	86.2%	--	78.3%	77.8%	59.0%	63.6%
Dog	70.4%	77.2%	80.4%	84.8%	--	83.1%	66.9%	67.5%
Cat	69.6%	77.5%	81.3%	84.7%	88.9%	--	65.5%	67.4%
Monkey	56.7%	59.6%	56.6%	57.0%	59.2%	58.4%	--	95.8%
Human	68.4%	74.6%	73.7%	63.1%	74.4%	75.3%	96.3%	--

## DNA HOMOLOGY

- 44 -

Data is presented as a cross-wise comparison of the ZPA protein and DNA sequences. The comparison of the protein sequences are shown in the upper right hand side of the table, above the diagonal dashed lines. The comparison of the DNA sequences are shown in the lower left hand side of the table,  
5 below the diagonal dashed lines. The ZPA DNA and deduced amino acid sequences are highly homologous between species. The homology is highest between members of the same order within the class *mammalia*. For example, the human and cynomolgus monkey (*primatea*), the pig and cow (*ungulata*), and the cat and dog (*carnivora*) sequences have the most  
10 similarity. The high degree of homology between the ZPA genes, as well as between the ZPB (see Example 14) and ZPC (Example 15) genes from a variety of mammalian species, implies a great deal of structural similarity in the ZP layers of these species. However, post-translational modification differences such as glycosylation and others, could represent a potential source  
15 of variation.

One protein processing site that all of these ZPA proteins have in common is a furin cleavage site (R-X-R/K-R; Hosaka *et al. J. Biol. Chem.*, 266:12127 (1991)) near the C-terminal end of the protein. In fact, with only a few exceptions, all ZP proteins contain a furin processing site near the C-  
20 terminus. This furin site could serve to cleave off a putative membrane anchor sequence which would allow the processed proteins to move toward the outer edge of the growing ZP layer.

The human ZPA gene contains an exon near the 3' end that is present in the cynomolgus monkey ZPA sequence, but not present in the ZPA genes from other species. This extra exon codes for an amino acid sequence that occurs after the furin processing site, which suggests that the C-terminal fragment generated by furin cleavage might still be important to the function of the ZP layer or to the oocyte in some way.  
25

There are 20 conserved cysteine residues and one or two non-  
30 conserved cysteine residues in each of the full-length ZPA sequences. The

- 45 -

non-conserved cysteine residues occur either in the N-terminal leader sequence region, or in the extreme C-terminal region of the sequence, where a large amount of the variation between the ZPA sequences occurs. The high degree of homology and the large number of conserved cysteine residues suggests that  
5 the tertiary structures of the ZPA proteins are similar.

It has been noted previously that there are regions of homology between the ZPA and ZPB class proteins (Schwoebel *et al.* *J. Biol. Chem.*, 266:7214 (1991); Lee *et al.* *J. Biol. Chem.*, 268: 12412 (1993); Yurewicz *et al.* *Biochem. Biophys. Acta* 1174:211 (1993)). Comparison of the human  
10 ZPA genomic structure with the human ZPB genomic structure shows these regions to be confined to exons 12, 13, and 14 of the human ZPA gene and exons 5, 6, and 7 of the human ZPB gene. This suggests that this homology might be due to a partial ancestral gene duplication. The ZPB proteins contain 21 conserved cysteine residues. The first 11 of these do not align  
15 with those in the ZPA proteins, but the last 10 match well. This extends the homology to approximately 270 amino acids, covering exons 11-16 of the ZPA gene and exons 4-9 of the ZPB gene, although the overall homology of the expanded region is slightly lower (approximately 43%). The remainder of the ZPA and ZPB genes show very little homology with each other, and the  
20 ZPC genes also show no extensive homology to the ZPA genes. In addition, the ZPA gene has no extensive sequence similarity to non-ZP nucleic acid and protein sequences in Genbank and the SwissProt data banks.

#### Example 14

##### Comparison of ZPB DNA and of Deduced Amino Acid Sequences

25 Table 6 shows the comparison of the six known ZPB DNA and protein sequences (the bovine and cynomolgus cDNA fragments are only compared to the corresponding regions of the other full-length ZPB sequences).

TABLE 6

## ZPB HOMOLOGY

## PROTEIN HOMOLOGY

	Rabbit	Bovine	Porcine	Feline	C. Monkey	Human
Rabbit	--	75.3%	65.3%	60.1%	70.2%	65.2%
Bovine	78.8%	--	82.3%	71.2%	69.9%	69.6%
Porcine	74.2%	86.2%	--	63.7%	63.6%	63.1%
Feline	69.5%	78.7%	72.9%	--	70.3%	64.6%
C. Monkey	78.9%	78.5%	78.2%	78.6%	--	92.3%
Human	74.3%	80.8%	73.3%	74.2%	95%	--

## DNA HOMOLOGY

- 47 -

The data are presented as cross-wise comparison of the ZPB protein and DNA sequences. The comparison of the protein sequences are shown in the upper right hand side of the table, above the diagonal dashed lines. The comparison of the DNA sequences are shown in the lower left hand side of the table, below the diagonal dashed lines.

5        The data shows considerable ZPB homology among members of different mammalian species. As was the case with ZPA, this homology is most pronounced between members of the same order within the class *mammalia*. For example, the human and cynomolgus monkey sequences (primata) and the pig and cow sequences (*ungulata*) have the most homology to each other. With only a few exceptions (noted below), the ZPB sequences show no homology to other DNA or protein sequences in the GenBank or 10      SwissProt databases. Hybridization experiments suggest that the ZPB transcripts are ovary specific.

15      Comparisons of the deduced amino acid sequences of the ZPB clones show more divergence within this genetic group than within the ZPA and ZPC groups. Comparison of the rabbit ZPB and porcine ZPB shows the sequences to be predominantly collinear (74% homologous) except that the rabbit has an additional upstream ATG codon which adds six codons to the 20      rabbit sequence.

25      The feline ZPB sequence has two additional amino acid inserts, which total 38 additional codons, in the first quarter of the gene, compared to the porcine and rabbit sequences. Both inserts occur just after cysteine residues, which suggests that if the cysteines are involved in disulfide bridges, these regions might form unique epitopes. However, the feline gene is still 73% homologous to porcine gene and 70% homologous to the rabbit gene.

30      The human gene has a sequence homologous to the first of the inserts in the cat sequence, but not the second. However, there are consensus splice site donor and acceptor sequences adjacent to this extra region in the human sequence, which if used would leave the coding sequence in frame.

Therefore, the sequence representing exon 2 could actually be two small exons (122 and 103 bp) separated by a small intron (84 bp). This would make the human sequence in this region identical to the pig sequence. The first extra region in the cat sequence is also flanked by in frame splice site donor and acceptor signals. If the extra region was removed from the cat sequence, it would differ from the pig sequence by only a single amino acid. However, the cat sequence was obtained from a cDNA clone made from an mRNA that appears to be fully processed. The second extra region in the cat sequence does not contain in frame splice site donor or acceptor signals, and therefore is probably not due to the presence of an unprocessed intron.

The cynomolgus monkey and human sequences have an additional seven codons at the C-terminus when compared to the other ZPB sequences. In the cynomolgus monkey, this is due to a two-base pair deletion, which causes a frameshift mutation which puts the termination codon used by the other species out of frame. The human sequence also contains this deletion, but in addition, there is also a base change that eliminates this termination codon.

There are 21 conserved cysteine residues in the ZPB proteins, the final 10 of which occur in a region that has homology to the ZPA proteins. This homology was noted previously (Schwoebel *et al.*, *supra*; Lee *et al.* *supra*, 1993; Yurewicz *et al.* *supra*, 1993), but examination of the genomic structure of the human ZPA and ZPB genes allowed the homology to be extended to approximately 270 amino acids. This homology could be due to a partial ancestral gene duplication. In addition to the conserved cysteine residues, the pig ZPB protein contains one additional cysteine residue in the putative leader sequence, and the human sequence contains four additional cysteine residues. The first of these is in the putative leader sequence (in a different location than pig), the second is in the region containing the additional insert, and the last two are in the C-terminal

- 49 -

extension caused by the mutated termination codon. These last two extra cysteine residues are conserved in the cynomolgus monkey sequence.

All of the ZP proteins contain a putative transmembrane domain near the C-terminus. However, the canonical furin proteolytic processing signal (R-X-R/K-R, Hosaka *et al. supra*, 1991), which occurs just prior to the transmembrane domain in all of the ZPA and ZPC proteins, is altered in the human (S-R-R-R), cynomolgus monkey (S-R-R-N) and rabbit (S-R-R-R) ZPB sequences. The significance of this is unknown, but it may indicate that these proteins are processed by a related system with specificity for di- or tribasic sequences, since the release of the putative transmembrane domain would be necessary for the ZPB protein to move as the ZP layer grows. There appears to be a great deal of proteolytic processing of the pig ZPA and ZPB (Yurewicz *et al. supra*,) proteins. There is no data concerning the post-translational modification of the ZPB proteins of cat, cow, cynomolgus monkey or human. The physiologic significance of this processing is unknown, but differential processing would present an avenue of variation among species of the highly conserved ZP proteins.

There is a question of whether humans actually transcribe the ZPB gene. Since the amount of human ovarian mRNA recovered was so small, there was not enough RNA to both construct a cDNA library and perform a Northern analysis. However, since cynomolgus monkey transcribes the ZPB gene, it is probable that the highly homologous human ZPB gene is also transcribed.

The apparent lack of a ZPB cDNA in the dog cDNA library is another puzzle. All of the libraries screened which contained any zona pellucida gene contained all three genes, except the dog. However, mRNA isolated from the ovary of a six-month old dog (the library was made from the ovary of a four-month old dog), includes a ZPB mRNA that comigrates with the porcine and cynomolgus monkey ZPB mRNA on a Northern blot. One possibility to explain the lack of a canine ZPB cDNA is that the transcriptional

- 50 -

timing of the three ZP genes is spread out, and since the ovary used to make the library was young, the transcription of the ZPB gene occurs later than the ZPA and ZPC genes (Andersen and Simpson, 1973).

**Example 15**

**5 Comparison of ZPC DNA and Deduced Amino Acid Sequences**

Table 7 shows the comparison of the DNA and deduced amino acid sequences from all of the ZPC cDNAs and genes.

TABLE 7

## ZPC HOMOLOGY

## PROTEIN HOMOLOGY

	Mouse	Hamster	Rabbit	Pig	Cow	Dog	Cat	Monkey	Human
Mouse	--	78.8%	65.9%	65.6%	64.0%	64.7%	63.3%	64.4%	67.0%
Hamster	84.7%	--	65.9%	65.6%	63.5%	65.1%	63.6%	68.2%	68.0%
Rabbit	70.1%	71.3%	--	68.2%	68.5%	65.3%	64.1%	59.4%	68.5%
Pig	71.5%	72.0%	74.6%	--	83.6%	75.7%	72.8%	69.2%	73.7%
Cow	70.5%	71.4%	74.5%	86.5%	--	74.5%	72.8%	67.4%	71.1%
Dog	70.1%	71.9%	71.5%	79.8%	80.3%	--	79.2%	66.5%	70.1%
Cat	70.9%	71.6%	73.0%	79.3%	80.0%	84.3%	--	71.1%	70.5%
Monkey	72.4%	74.1%	71.3%	76.6%	77.2%	73.8%	77.8%	--	90.6%
Human	74.1%	75.0%	76.2%	80.0%	79.6%	77.7%	78.8%	94.4%	--

## DNA HOMOLOGY

- 52 -

The data are presented as a cross-wise comparison of the ZPC protein and DNA sequences. The comparison of the protein sequences are shown in the upper right hand side of the table, above the diagonal dashed lines. The comparison of the DNA sequences are shown in the lower left hand side of the table, below the diagonal dashed lines.

ZPC proteins and DNA sequences show a higher degree of homology than the ZPA and ZPB DNAs and proteins. As was the case with ZPA and ZPB, the homology is most pronounced in members of the same order within the class *mammalia*; the human and cynomolgus monkey sequences (*primate*), the cat and dog sequences (*carnivora*), the pig and cow sequences (*ungulata*), and the mouse and hamster sequences (*rodenta*). The ZPC transcripts are ovary specific, based on Northern blot analysis and comparison to the sequences in the GenBank and SwissProt databases detects no significant non-ZP homology. Comparison of the deduced amino acid sequences of the known ZPC genes detects three regions that contain large numbers of non-consensus sequences. These regions are: the putative leader sequences and the first 20-25 amino acids of the mature protein; the region containing the peptide that was identified as a sperm-binding region in the mouse (Millar *et al.* *Science* 216:935-938 (1989)); and the C-terminal region of the proteins that might be removed from the mature protein at the furin processing site (see below).

The epitope identified as a putative sperm-binding site (Millar *et al. supra*, 1989) occurs immediately before a furin proteolytic cleavage site (Hosaka *et al.*, 1991). The furin site (R-X-R/K-R) is highly conserved in all of the ZPC sequences. However, it should be noted that the canine ZPC sequence contains a second furin site, 19 amino acids upstream from the first furin site. Also as is the case with ZPA and ZPB, cleavage by furin of the ZPC proteins would remove a putative membrane anchor sequence (Klein *et al.*, 1985), which would allow the processed ZPC protein to move toward the outer layer of the expanding oocyte. Therefore, this sperm-binding site

- 53 -

probably represents the C-terminus of the mature proteins. However, there is very little homology (even between hamster and mouse) in the regions of the ZPC proteins corresponding to this epitope. This might indicate that this region contributes to the species specificity of sperm-egg binding.

5           The variation that is seen at the C-terminus of the ZPC proteins occurs in the putative transmembrane region. This variation could indicate that this amino acid sequence is less important than the overall hydrophobicity of the amino acids in this region, similar to the lack of homology seen in leader sequences. However, it is also possible that this variation signifies a  
10          species-specific function for this region.

15          Each ZPC sequence contains 14 conserved cysteine residues, but each sequence also has one or two extra cysteine residues that are shared only with one or a few other sequences. These extra cysteine residues are near the N- or C-terminus of the proteins, where the greatest sequence variation exists. However, the large number of conserved cysteine residues probably indicates that the overall structure of the central core of all of these proteins is quite conserved.

#### Example 16

#### Immunization of Cynomolgus Monkeys With HSPZ

20          A sexually mature cynomolgus monkey was immunized with HSPZ to test the ability of HSPZ to induce infertility. HSPZ was prepared as described in Example 6. HSPZ was mixed with the following GMDP/oil adjuvant. 50 µg GMDP (N-acetyl-D-glucosaminyl-( $\beta$ 1-4)-N-acetylmuramyl-D-isoglutamine) (CC. Biotech, Poway, CA); 42.1 of mineral oil, 15.8% pluronic  
25          VC-121 (block polymer polyols, BASF-Wyandotte, Parsippany, NJ). The animal received a series of 4 subcutaneous injections of 1 mg of HSPZ in the GMDP/oil adjuvant beginning with a priming dose followed four weeks later by a booster dose, which was followed by two booster doses five weeks apart

- 54 -

which were followed six weeks later by a final dose. This dosage regimen resulted in an anovulatory monkey having antibody titers against its cynomolgus monkey heat-solubilized zona pellucida prepared as described for HSPZ. The peak antibody titers to cynomolgus monkey HSPZ were 1:8000-  
5 1:16,000.

A fractionated preparation of HSPZ which is essentially native porcine ZPA and ZPB was prepared by isoelectric focusing, as described in Example 6 and was used to vaccinate cynomolgus monkeys using 1 mg of fractionated HSPZ in GMDP/oil injected subcutaneously according to the  
10 following schedule: a priming dose was given followed approximately 6 weeks later by a booster dose followed by a final booster dose 11 weeks after the previous booster dose. The immunized monkeys achieved peak antibody titers of 1:4,000-1:8,000 against monkey heat-solubilized zona pellucida while maintaining a regular ovulatory cycle. However, despite maintaining a  
15 regular ovulatory cycle, the monkeys remained infertile until their antibody titers to monkey heat-solubilized zona pellucida fell below 1:500 after which the animals became pregnant upon breeding.

Immunization of cynomolgus monkeys with recombinant baculovirus produced canine ZPC and porcine ZPC (prepared as described in  
20 Example 18) failed to induce infertility despite inducing antibody production against monkey heat-solubilized zona pellucida. One possible explanation for this is that the glycosylation pattern of ZP proteins produced in the baculovirus system may prevent recognition of the epitopes responsible for induction of infertility.

25 Bacterially produced porcine ZPA, ZPB, and ZPC described above administered to cynomolgus monkeys failed to induce detectable antibody titers against cynomolgus monkey heat-solubilized zona pellucida even though antibody titers against the presented antigens were produced.

- 55 -

#### Example 17

#### Mapping of Mammalian Zona Pellucida Protein Epitopes

A Pin Technology™ Epitope Scanning Kit purchased from Chiron Mimotopes U.S., Emeryville, CA (Catalog No. PT-02-20000A) was used for mapping epitopes in Zona Pellucida proteins. The procedures described in the kit manual were followed, with the exception of modifications in the ELISA testing procedure (described below).

Briefly, Pin Technology software was installed in a United Business Machines 486/33 computer according to the manufacturer's instructions. The protein sequence was entered into the computer program, the desired peptide length, and degree of overlap between peptides were selected, and a protocol containing the daily requirements of activated protected amino acid derivatives and their location in the coupling tray wells was printed. Prior to use, the pins were first washed once with dimethylformamide (DMF), and then with methanol three times, each wash lasting for two minutes. The pin block was air dried and the pins were deprotected by agitation in a 20% mixture of piperidine in DMF at room temperature for 30 minutes. The pins were washed again as described above, except that the washes were for 5 minutes each, and the pin block was then air dried. The required amino acid derivative solutions were prepared and dispensed into the wells of the synthesis tray according to the protocol for the current cycle. The dried mimotope pins were washed once more in a DMF bath for 5 minutes and then positioned appropriately in the wells of the synthesis tray. The assembly was then sealed in a plastic bag and incubated at 30°C for approximately 22 hours. On the following day, the pin block was removed from the coupling tray and subjected to the same cycle of washing, deprotection, and coupling steps as outlined above; however, using the amino acid derivatives and their tray location appropriate to the next cycle. The

- 56 -

foregoing cycle of washing, deprotection, washing, and coupling was repeated until the peptide sequences were completed.

After coupling the terminal amino acids of the peptides, the pin block was washed, air dried, deprotected, washed and air dried as before.

5      The terminal amino groups of the peptides were then acetylated by immersion of the pins in a mixture containing 5 parts DMF, 2 parts acetic anhydride, and 1 part triethylamine, by volume, dispensed in the wells of a polypropylene coupling tray, and incubating at 30°C for 90 minutes. The pin block was removed, subjected to another washing sequence as before, and air dried.

10     Side chain deprotection of the peptides was performed by agitating the pin block in a mixture containing 95 parts trifluoroacetic acid, 2.5 parts anisole, and 2.5 parts ethanedithiol, by volume, at room temperature for 4 hours. The pin block was then air dried for approximately 10 minutes, sonicated in a bath containing 0.1% hydrochloric acid in a mixture containing 15 equal parts of methanol and deionized water, by volume, for 15 minutes, and finally air dried.

Prior to ELISA testing, the pins were subjected to a disruption procedure involving sonication in a bath consisting of a mixture containing 39 parts sodium dihydrogen orthophosphate, 25 parts sodium dodecyl sulfate, 0.1 20 part 2-mercaptoethanol, and 2500 parts deionized water, by weight, adjusted to pH 7.2 with 50% sodium hydroxide solution. The sonication was performed at 55 to 60°C for approximately 45 minutes. The pin block was then washed by immersion with gentle agitation in three sequential baths of deionized water at 60 degrees for three minutes each. Finally, the pin block 25 was immersed in gently boiling methanol for approximately 4 minutes and then air dried.

#### Preparation of Antisera

Antisera directed against zona pellucida proteins was prepared by immunizing the appropriate animals with the appropriate zona pellucida

- 57 -

protein using procedures well known in the art and described in E. Harlow and D. Lane in *Antibodies, A Laboratory Manual*, Chapter 5, Cold Spring Harbor Laboratory, 1988 which is incorporated herein by reference. Biotinylated antisera was prepared by a modification of the procedure 5 described in Harlow *supra* (page 314). Briefly, to a solution containing between 1 and 3 mg per ml of the selected antibody IgG fraction in phosphate buffer with saline (PBS) at pH 7.2 was added a solution containing 25 to 250 micrograms biotinamidocaproate, N-hydroxysuccinimide ester (Sigma, Cat No. B2643) in dimethyl sulfoxide at a concentration of 10 mg/ml. The 10 mixture was mixed well and then incubated at room temperature for 4 hours. One molar ammonium chloride solution in the amount corresponding to 20 microliters per 250 micrograms biotin ester was added, and the resulting mixture was incubated at room temperature for 10 minutes. Unreacted biotin ester was then removed by extensive diafiltration with PBS using a Centricon- 15 30 (TM) microconcentrator devices (Amicon Division, W.R. Grace & Co., Inc., Beverly MA). The dilution factor for the resulting conjugate was determined by ELISA titration against the appropriate native protein.

#### ELISA Testing

A modification of the procedure described in the Epitope 20 Scanning Kit manual was employed.

After disruption, the mimotope pins were blocked by incubation with "supercocktail" (10 g ovalbumin, 10 g bovine serum albumin, and 1 ml Tween 20 detergent per liter of PBS) at room temperature for 1 hour. This was followed by incubation at room temperature for 2 hours with 25 appropriately diluted biotinylated antisera. The pins were washed 4 times with PBS containing 0.5% Tween 20 (PBST) at room temperature for 10 minutes each time, with agitation.

The pins were then incubated at room temperature for 1 hour with the secondary antibody, horseradish peroxidase-streptavidin conjugate

- 58 -

(Zymed Laboratories, Inc., South San Francisco, CA) diluted 1:2500 with PBST. They were washed again as described above.

Substrate buffer was prepared by combining 200 ml 1.0 M. disodium hydrogen orthophosphate solution with 160 ml 1.0 M. citric acid solution, diluting the mixture with 1640 ml deionized water, and adjusting to pH 4.0 using either citric acid or sodium hydroxide solutions. Substrate solution was prepared by dissolving 10 mg 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt in 20 ml substrate buffer and adding 6 microliters 30% hydrogen peroxide. The mimotope pins were 5 incubated at room temperature with this solution, using microtiter plates containing 150 microliters per well. When color development appeared to be appropriate for measurement by an ELISA plate reader, the pin block was removed and the plate was read at a wavelength of 450 nm. The pin block 10 was then disrupted by the procedure described above.

15 The data were entered into the Pin Technology™ computer program, which performed statistical analysis and evaluation and furnished a print-out of the results identifying the strongest binding epitopes. Briefly, the 25% of the wells having the lowest optical density readings were assumed to represent background in each experiment. The mean value and the standard 20 deviation of these readings were calculated. Significant recognition of peptides by antisera was attributed to the pins corresponding to those wells showing absorbance readings greater than the sum of the background mean and three standard deviations from the mean.

Human ZPA epitopes were examined for reactivity with mouse 25 anti-human ZP antiserum prepared as described above. Peptides of 15 amino acids in length were synthesized beginning with amino acid number 1 as illustrated in SEQ ID NO. 43. Successive peptides having a 7-amino acid overlap with the preceding peptide of the series were synthesized. The following peptides were shown to bind mouse anti-human ZP antiserum: 1- 30 15, 9-23, 25-39, 33-47, 65-79, 81-95, 89-103, 97-111, 105-119, 113-127,

- 59 -

121-135, 129-143, 145-159, 153-167, 161-175, 193-207, 209-223, 217-231,  
225-239, 241-255, 249-263, 273-287, 281-295, 289-303, 305-319, 313-327,  
321-335, 329-343, 337-351, 345-359, 385-399, 393-407, 401-415, 409-423,  
417-431, 425-439, 441-455, 449-463, 457-471, 481-495, 489-503, 497-511,  
5 505-519, 513-527, 521-535, 537-551, 545-559, 561-575, 569-583, 577-591,  
585-599, 601-615, 609-623, 617-631, 625-639, 633-647, 641-655, 665-679,  
697-711, 705-719, 713-727, 721-735, and 729-743.

Similarly, human ZPB epitopes were mapped using mouse anti-human ZP antiserum. In these experiments, 15 amino acid peptides were  
10 synthesized beginning with amino acid number 1 as set out in SEQ ID NO. 41. The overlap between successive peptides in this case was 9 amino acids. The following peptides were shown to bind mouse anti-human ZP antiserum:  
7-21, 25-39, 31-45, 49-63, 67-81, 73-87, 79-93, 91-105, 103-117, 121-135,  
193-207, 205-219, 211-225, 217-231, 223-237, 229-243, 253-267, 259-273,  
15 265-279, 283-297, 289-303, 295-309, 301-315, 307-321, 313-327, 319-333,  
343-357, 349-363, 355-369, 367-381, 373-387, 379-393, 385-399, 403-417,  
409-423, 415-429, 421-435, 433-447, 439-453, 445-459, 451-465, 481-495,  
487-501, 499-513, 505-519, 511-525, 523-537, 529-543, and 547-561.

Human ZPC epitopes were mapped using mouse anti-human ZP antiserum. In these experiments, the 15 amino acid peptides were synthesized  
20 beginning with amino acid number 1 as set out in Chamberlin *et al.*, *Proc. Nat'l Acad. Sci. USA* 87:6014-6018 (1990) which is incorporated herein by reference. The overlap between successive peptides was 10 amino acids. The following peptides were shown to bind mouse anti-human ZP antiserum: 21-  
25 35, 51-65, 116-130, 146-160, 151-165, 181-195, 241-255, 251-265, 271-285,  
296-310, 321-335, 401-415, and 411-425.

Canine ZPC epitopes were mapped using rabbit anti-canine ZP antiserum. In these experiments, the 15 amino acid peptides were synthesized  
30 beginning at amino acid number 1 set out in SEQ ID NO. 10. The overlap between successive peptides was 5 amino acids. The following peptides were

- 60 -

shown to bind rabbit anti-canine ZP antiserum: 51-65, 61-75, 81-95, 131-145, 181-195, and 301-315.

Feline ZPC epitopes were mapped using rabbit anti-feline ZP antiserum. In these experiments, the 15 amino acid peptides were synthesized beginning at amino acid number 1 as set out in SEQ ID NO. 18. The overlap between successive peptides was 5 amino acids. The following peptides were shown to bind rabbit anti-feline ZP: 36-50, 46-60, 56-70, 76-90, 96-110, 106-120, 116-130, 126-140, 136-150, 146-160, 156-170, 186-200, 196-210, 246-260, 266-280, 276-290, 286-300, 296-310, 316-330, 326-340, 336-350, 10 346-360, 376-390, 396-410, and 406-420.

Bovine ZPC epitopes were mapped using rabbit anti-bovine ZP antiserum. In these experiments, the overlapping 15 amino acid peptides were synthesized beginning at amino acid number 1 as set out in SEQ ID NO. 24. The overlap between peptides was 10 amino acids. The following peptides 15 were shown to be reactive with rabbit anti-bovine ZP antiserum: 1-15, 31-45, 51-65, 56-70, 61-75, 76-90, 106-120, 111-125, 116-130, 121-135, 131-145, 136-150, 141-155, 146-160, 151-165, 161-175, 181-195, 186-200, 191-205, 196-210, 201-215, 206-220, 216-230, 226-240, 241-255, 246-260, 261-275, 266-280, 271-285, 276-290, 291-305, 296-310, 301-315, 316-330, 321-335, 20 326-340, 331-345, 336-350, 341-355, 356-370, 361-375, 376-390, 381-395, 386-400, 396-410, 401-415, and 406-420.

#### Example 18

##### Immunization of Dogs with Recombinant ZPC Proteins

Dogs were immunized with various preparations of recombinant 25 canine ZPC. The plasmid pZ169 bacterial expression vector (Figure 5) was constructed as follows. The parent vector pZ98 (described in Example 9) was digested with the restriction enzymes *Pvu*I and *Bam* HI, and the large

- 61 -

fragment was gel purified. Into this vector was ligated a fragment created by annealing the following oligonucleotides:

5' CGCCCTTCCCAGCAACTGCACCATCACCAACCATGGG 3'  
(SEQ ID NO. 50); and

5 5' GATCCCCATGGTGGTGGTGTGGCAGTTGCTGGGAAGGGCGAT 3'  
(SEQ ID NO. 51).

These oligonucleotides create a fragment with *Pvu*I and *Bam*HI ends, and codes for the hexapeptide sequence His<sub>6</sub>. This intermediate vector was digested with the restriction enzymes *Bam*HI and *Eco*RI, and the large  
10 fragment was gel purified. Into this vector was ligated a fragment created by annealing the following oligonucleotides:

5' GATCCCTCGAGCCACCATCACCAACCATCATG 3'  
(SEQ ID NO. 52); and

15 5' AATTCCATGATGGTGGTGTGGCAGTTGCTGGAGG 3'  
(SEQ ID NO. 53).

These oligonucleotides create a fragment with *Bam*HI and *Eco*RI ends and an *Xba*I site just downstream of the *Bam*HI site, and which codes for the hexapeptide sequence His<sub>6</sub>. This new vector was named pZ88, and contains unique *Bam*HI and *Xba*I cloning sites between two His<sub>6</sub> sequences. To create  
20 pZ169, the pZ88 vector was digested with the restriction enzymes *Bam*HI and *Xba*I, and the large fragment was gel purified. Into this vector was ligated a fragment generated by performing a PCR (polymerase chain reaction) of the canine ZPC cDNA using the following oligonucleotides:

- 62 -

5' CCCGGATCCGCAGACCATCTGCCAACTGAG 3'  
(SEQ ID NO. 54); and

5' GCGCTCGAGGGCATATGGCTGCCAGTGTG 3'  
(SEQ ID NO. 55).

5 This PCR creates a fragment containing amino acids 23-207 of the canine ZPC sequence, with *Bam*HI and *Xba*I ends. This new vector is named pZ169, (Figure 5) and produces a protein containing amino acids 1-56 of the *E. coli*  $\beta$ -galactosidase sequence, His<sub>6</sub>, amino acids 23-207 of the canine ZPC sequence, His<sub>6</sub>, and amino acids 1006-1023 of the *E. coli*  $\beta$ -galactosidase sequence. This protein is referred to as N-terminal canine ZPC. In Figure 10 5, pTAC refers to the tac promoter described above; AmpR refers to an ampicillin resistance marker, ori is an *E. coli* origin of replication sequences and pLaci is the lacI promoter which drives expression of the lacI gene.

15 Recombinant canine ZPC was produced and purified as described in Example 9. A baculovirus expression vector pZ145 was constructed as follows. The parent vector pBlueBac2 (purchased from Invitrogen Corporation, San Diego, CA) was digested with the restriction enzymes *Nhe*I and *Bam*HI, and the large fragment was gel purified. Into this vector was ligated a fragment generated by a PCR of the porcine ZPC cDNA 20 using the following oligonucleotide:

5' CGCGCTAGCAGATCTATGGCGCCGAGCTGGAGGTTTC 3'  
(SEQ ID NO. 56); and

5' CGCGGATCCTATTAAATGGTGGTGATGGTGGTGACTAGTGGACCCTTCCA 3'  
(SEQ ID NO. 57).

25 This PCR creates a fragment with *Nhe*I and *Bam*HI ends, and contains amino acids 27-350 of the porcine ZPC sequence followed by an *Spe*I site and the hexapeptide His<sub>6</sub>. This new vector is named pZ147. To create the pZ145 vector, pZ147 is digested with *Nhe*I and *Spe*I and the large fragment is gel purified (this removes the pig ZPC sequence). Into this vector was ligated a

- 63 -

fragment generated by a PCR of the canine ZPC cDNA using the following oligonucleotides:

5' CCCGCTAGCAGATCTATGGGCTGAGCTATGGAATTTC 3'  
(SEQ ID NO. 58); and

5 5' CGCACTAGTTGACCCCTCTATACCATGATCACTA 3'  
(SEQ ID NO. 59).

This PCR creates a fragment with *NheI* and *SpeI* ends, and contains amino acids 1-379 of the canine sequence. Transformants of this ligation were screened for the presence of the inserted *NheI/SpeI* fragment in the correct orientation (since the *NheI* and *SpeI* sticky ends are identical). This new vector is named pZ145, (Figure 6) and produces a protein containing amino acids 1-379 of the DZPC sequence followed by His. This protein is referred to as baculo-canine ZPC. In Figure 6, pP represents the baculovirus polyhedrin promoter, AmpR represents an ampicillin resistance marker, LacZ represents the gene for  $\beta$ -galactosidase, pE is a constitutive promoter which drives the expression of LacZ and ori is the *E. coli* origin of replication.

Recombinant baculovirus derived canine ZPC was produced by co-transfected insect SF9 cells with pZ145 and *Autographica californica* multiply enveloped nuclear polyhedrosis virus (AcMNPV) using methods well known in the art as described in the MAXBAC™ kit purchased from Invitrogen, San Diego, CA. Recombinant canine ZPC produced in SF9 cells was prepared from cotransfected SF9 cells as follows. Cotransfected cells were harvested and pelleted by centrifugation and recombinant canine ZPC was purified as was described in Example 9 for purification from a cell pellet. Recombinant canine ZPC may also be isolated from the culture medium and purified on a Ni-column as described in Example 9.

Other expression vectors which are capable of expressing zona pellucida encoding nucleotide sequences under the control of a variety of

- 64 -

regulatory sequences are within the scope of the present invention and are readily constructed using methods well known in the art.

Recombinant zona pellucida proteins may also be modified to increase their potential antigenicity by a variety of methods well known in the art. For example, a recombinant dog ZPC was modified by palmitylation was prepared as follows. Approximately 1 mg of recombinant ZPC produced using the plasmid pZ169 as described above was brought to a final concentration of 8M urea (total volume 0.2-0.3 mls.). A palmitylation solution (PI<sub>2</sub>O/TEA) was then prepared by adding palmitic anhydride to triethylamine to give a final concentration of palmitic anhydride of 20 mg/ml of triethylamine.

Approximately 10 µl of PI<sub>2</sub>O/TEA solution was added to 1 mg of recombinant canine ZPC in 8M urea (described above). The mixture was allowed to stand at room temperature for a least two hours after which the preparation was ready for mixture with GMDP/oil adjuvant.

Chitosan modification is another useful modification of canine ZPC for the practice of the present invention. Briefly, 1.5 ml of sterile mineral oil was added to 1.5 ml of recombinant canine ZPC solution prepared as described above using the plasmid pZ169 (2 mg/ml ZPC, 3 mg total is 8M urea) was mixed with 5 drops of Arlacel A (mannide monooleate, Sigma, St. Louis, MO). Subsequently, 0.75 ml of Chitosan (2% wt/vol. is 0.5M sodium acetate, pH 5.0) was added, and the mixture was sonicated for 10-20 seconds, followed by the addition of 0.045 ml of 50% NaOH and another round of sonication for 10-20 seconds. Finally, 10µl of 10 mg/ml GMDP/8M urea was added.

A group of three dogs was immunized five times each at one-month intervals with subcutaneous injections of 1 mg doses of the N-terminal canine ZPC modified by the addition of chitosan prepared as described above. Immunized dogs developed antibody titers of 1:8000-30 1:16000 against heat solubilized dog zona pellucida (self-titers) using methods

- 65 -

described above. The estrus cycle of the dogs showing self-titers was anovulatory and prolonged (4-6 weeks instead of the normal 10-day to 14-day cycle for normal dogs). Of the three immunized dogs, two have experienced their first estrus; one of the two dogs exhibited estrus six months after the first 5 immunization and was bred and found to be infertile. The second of the two dogs experienced estrus and remained infertile nine months after the first immunization. The third dog has yet to experience estrus more than nine months after immunization.

Another group of four dogs were immunized three times at one-month intervals using 1 mg doses of palmitoylated canine ZPC (prepared as described above) in GMDP/oil adjuvant administered subcutaneously. These animals achieved self-titers (against heat solubilized dog zona pellucida) of 1:4000-1:8000. Nearly seven months after immunization, two of the four dogs experienced estrus and remain infertile. The remaining two dogs have 15 yet to experience estrus.

Another set of dogs was immunized 3 times at one-month intervals, using subcutaneous injections of 1 mg of recombinant canine ZPC produced using pZ166, (a plasmid similar to pZ169 but containing a DNA sequence encoding amino acids 23-379 of the canine ZPC protein) in 20 GMDP/oil adjuvant. These animals failed to develop self-titers and became pregnant after breeding. Similarly, dogs immunized with canine ZPC fragments produced using the baculovirus system failed to induce infertility.

#### Example 19

Vaccination of Cows and Cats with  
25 Recombinant Zona Pellucida Proteins

Preliminary studies were undertaken to assess the ability of recombinant zona pellucida proteins to induce infertility in cows and cats.

- 66 -

Cows were injected with 3 or more doses (in GMDP (250 µg) oil adjuvant) of 1 mg of a variety of recombinantly derived ZPC proteins from canine and porcine sources including canine ZPC produced using the plasmid pZ169 as shown in Figure 5. Recombinant proteins were administered in an unmodified form and in palmitoylated and chitosan modified forms. None of the ZP protein preparations induced self-titers or infertility in the vaccinated cows. Further studies are underway using different recombinant preparations of zona pellucida proteins and differing dosage regimens in attempts to induce self-titers and infertility in cows.

Similarly, cats were vaccinated with the following recombinant zona pellucida proteins: a mixture of recombinant feline ZPA, ZPB, and ZPC; porcine ZPC produced using pZ156 as described above and shown in Figure 3; and canine ZPC produced using the plasmid pZ169 described above and shown in Figure 5. Cats vaccinated using these ZP protein preparations produced antibody to the vaccine proteins, but produced no self-titers and were consequently fertile. Studies are ongoing to determine the effects of modifying the recombinant zona pellucida proteins in attempts to stimulate the production of self-titers and to induce infertility.

Studies are also ongoing to select other recombinantly derived zona pellucida protein fragments for testing as possible immunocontraceptives.

Numerous modifications in variations in the practice of the invention as illustrated in the above examples are expected to occur to those of ordinary skill in the art. Consequently, the illustrative examples are not intended to limit the scope of the invention as set out in the appended claims.

- 67 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) ADDRESSEE: ZONAGEN, Inc.  
(B) STREET: 2408 Timberloch Place, B-4  
(C) CITY: The Woodlands  
(D) STATE: Texas  
(E) COUNTRY: United States of America  
(F) POSTAL CODE: 77380

(A) ADDRESSEE: Harris Ph.D., Jeffrey D.  
(B) STREET: 15 Flatstone  
(C) CITY: The Woodlands  
(D) STATE: Texas  
(E) COUNTRY: United States of America  
(F) POSTAL CODE: 77381

(A) ADDRESSEE: Hsu, Kuang T.  
(B) STREET: 71 N. Misty Morning Trace  
(C) CITY: The Woodlands  
(D) STATE: Texas  
(E) COUNTRY: United States of America  
(F) POSTAL CODE: 77381

(A) ADDRESSEE: Podolski, Joseph S.  
(B) STREET: 3 Pebble Hollow Court  
(C) CITY: The Woodlands  
(D) STATE: Texas  
(E) COUNTRY: United States of America  
(F) POSTAL CODE: 77381

(ii) TITLE OF INVENTION: Materials and Methods for Immunocontraception

(iii) NUMBER OF SEQUENCES: 59

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun  
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive  
(C) CITY: Chicago  
(D) STATE: Illinois  
(E) COUNTRY: United States of America  
(F) POSTAL CODE: 60606-6402

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(B) FILING DATE: 09-NOV-1992

(ix) ATTORNEY/AGENT INFORMATION:

- 68 -

(A) NAME: Clough, David W.  
 (B) REGISTRATION NUMBER: 36,107  
 (C) REFERENCE/DOCKET NUMBER: 31745

(ix) TELECOMMUNICATION INFORMATION:  
 (A) TELEPHONE: 312/474-6653  
 (B) TELEFAX: 312/474-0448  
 (C) TELEX: 25-3856

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2214 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Sus scrofa  
 (D) DEVELOPMENTAL STAGE: Juvenile  
 (E) HAPLOTYPE: Diploidy  
 (F) TISSUE TYPE: Ovary  
 (G) CELL TYPE: Oocyte

(ix) FEATURE:  
 (A) NAME/KEY: sig\_peptide  
 (B) LOCATION: 12..119

(ix) FEATURE:  
 (A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 120..2153

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 12..2153

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCGGG C AGG CAC AGA GGA GAC AGT GGG AGA CCC TTA AGC TGG CTC	50
Arg His Arg Gly Asp Ser Gly Arg Pro Leu Ser Trp Leu	
-36 -35 -34 -33 -32 -31 -30 -29 -28 -27 -26 -25	-25
AGT GCA AGC TGG AGG TCA CTT CTT CTA TTT TTC CCC CTT GTG ACT TCA	98
Ser Ala Ser Trp Arg Ser Leu Leu Phe Pro Leu Val Thr Ser	
-20 -19 -18 -17 -16 -15 -14 -13 -12 -11 -10	-10
GTG RAC TCC ATA GGT GTC AAT CAG TTG GTG AAT ACT GCC TTC CCA GGT	146
Val Asn Ser Ile Gly Val Asn Gln Leu Val Asn Thr Ala Phe Pro Gly	
-5 -4 -3 -2 -1 0 1 2 3 4 5	5
ATT GTC ACT TGC CAT GAA AAT AGA ATG GTC GTG GAA TTT CCA AGA ATT	194
Ile Val Thr Cys His Glu Asn Arg Met Val Val Glu Phe Pro Arg Ile	
10 15 20 25	25
CTT GGC ACT AAG ATA CAG TAC ACC TCT GTG GTG GAC CCT CTT GGT CTT	242
Leu Gly Thr Lys Ile Gln Tyr Thr Ser Val Val Asp Pro Leu Gly Leu	
30 35 40	40
GAA ATG ATG AAC TGT ACT TAT GTT CTG GAC CCA GAA AAC CTC ACC CTG	290

- 69 -

Glu Met Met Asn Cys Thr Tyr Val Leu Asp Pro Glu Asn Leu Thr Leu 45 50 55	
AAG GCC CCA TAT GAA GCC TGT ACC AAA AGA GTG CGT GGC CAT CAC CAA Lys Ala Pro Tyr Glu Ala Cys Thr Lys Arg Val Arg Gly His His Gln 60 65 70	338
ATG ACC ATC AGA CTC ATA GAT GAC AAT GCT GCT TTA AGA CAA GAG GCT Met Thr Ile Arg Leu Ile Asp Asp Asn Ala Ala Leu Arg Gln Glu Ala 75 80 85	386
CTC ATG TAT CAC ATC AGC TGT CCT GTT ATG GGA GCA GAA GCC CCT GAT Leu Met Tyr His Ile Ser Cys Pro Val Met Gly Ala Glu Gly Pro Asp 90 95 100 105	434
CAG CAT TCG GGA TCC ACA ATC TGC ATG AAA GAT TTC ATG TCT TTT ACC Gln His Ser Gly Ser Thr Ile Cys Met Lys Asp Phe Met Ser Phe Thr 110 115 120	482
TTT AAC TTT TTT CCC CGG ATG CCT GAC GAA AAT GTG AAA CGT GAG GAT Phe Asn Phe Pro Gly Met Ala Asp Glu Asn Val Lys Arg Glu Asp 125 130 135	530
TCG AAG CAG CGC ATG GGA TGG AGC CTT GTA GTT GGT GAC GGT GAA AGA Ser Lys Gln Arg Met Gly Trp Ser Leu Val Val Gly Asp Gly Glu Arg 140 145 150	578
CCC CGA ACT CTG ACC TTT CAG GAG GCC ATG ACC CAA CGA TAT AAT TTC Ala Arg Thr Leu Thr Phe Gln Glu Ala Met Thr Gln Gly Tyr Asn Phe 155 160 165	626
CTG ATA GAG AAC CAG AAG ATG AAC ATC CAA GTG TCA TTC CTT GCC ACT Leu Ile Glu Asn Gln Lys Met Asn Ile Gln Val Ser Phe His Ala Thr 170 175 180 185	674
GGG GTG ACT CGC TAC TCG CAA GGT AAC AGT CAT CTC TAC ATG GTA CCT Gly Val Thr Arg Tyr Ser Gln Gly Asn Ser His Leu Tyr Met Val Pro 190 195 200	722
CTG AAG CTT AAA CAT GTA TCT CAT GGG CAG TCT CTC ATC TTA GCA TCA Leu Lys Leu Lys His Val Ser His Gly Gln Ser Leu Ile Leu Ala Ser 205 210 215	770
CAA CTC ATC TGT GTG GCA GAT CCT GTG ACC TGT AAT GCC ACA CAC GTG Gln Leu Ile Cys Val Ala Asp Pro Val Thr Cys Asn Ala Thr His Val 220 225 230	818
ACT CTT GCC ATA CCA GAG TTT CCT GGG AAG CTA AAA TCC GTG AAC TTG Thr Leu Ala Ile Pro Glu Phe Pro Gly Lys Leu Lys Ser Val Asn Leu 235 240 245	866
GGA ACT GGG AAT ATT GCT GTG AGC CAG CTG CAC AAA CAC GGG ATT GAA Gly Ser Gly Asn Ile Ala Val Ser Gln Leu His Lys His Gly Ile Glu 250 255 260 265	914
ATG GAA ACA ACA AAC GCC CTG AGG TTG CAT TTC AAC CAA ACT CTT CTC Met Glu Thr Thr Asn Gly Leu Arg Leu His Phe Asn Gln Thr Leu Leu 270 275 280	962
AAA ACA RAT GTC TCT GAA AAA TGC CTA CCA CAT CAG TTG TAC TTA TCT Lys Thr Asn Val Ser Glu Lys Cys Leu Pro His Gln Leu Tyr Leu Ser 285 290 295	1010
TCA CTC AAG CTG ACT TTT CAC AGT CAA CTA GAG GCA GTA TCC ATG GTG Ser Leu Lys Leu Thr Phe His Ser Gln Leu Glu Ala Val Ser Met Val 300 305 310	1058

- 70 -

ATT TAT CCT GAG TGT CTC TGT GAG TCA ACA GTC TCT TTA GTT TCA GAG ile Tyr Pro Glu Cys Leu Cys Glu Ser Thr Val Ser Leu Val Ser Glu 315 320 325	1106
GAG CTA TGC ACT CAG GAT GGG TTT ATG GAC GTC AAG GTC CAC AGC CAC Glu Leu Cys Thr Gln Asp Gly Phe Met Asp Val Lys Val His Ser His 330 335 340 345	1154
CAA ACA AAA CCA GCT CTC AAC TTG GAT ACC CTC AGG GTG GGA GAC TCA Gln Thr Lys Pro Ala Leu Asn Leu Asp Thr Leu Arg Val Gly Asp Ser 350 355 360	1202
TCC TGC CAG CCA ACC TTT AAA GCT CCA GCT CAG GGG CTG GTA CAG TTT Ser Cys Gln Pro Thr Phe Lys Ala Pro Ala Gln Gly Leu Val Gln Phe 365 370 375	1250
CGC ATA CCC CTG AAT GGA TGT GGA ACA AGA CAT AAG TTC AAG AAT GAC Arg Ile Pro Leu Asn Gly Cys Gly Thr Arg His Lys Phe Lys Asn Asp 380 385 390	1298
AAA GTC ATC TAT GAA AAT GAA ATA CAT GCT CTC TGG GCA GAT CCT CCA Lys Val Ile Tyr Glu Asn Gln Ile His Ala Leu Trp Ala Asp Pro Pro 395 400 405	1346
AGC GCC GTT TCC AGA GAT AGT GAG TTC AGA ATG ACA GTG AGG TGC TCT Ser Ala Val Ser Arg Asp Ser Glu Phe Arg Met Thr Val Arg Cys Ser 410 415 420 425	1394
TAC AGC AGC AGC AAC ATG CTA ATA AAT ACC AAT GTT GAA AGT CTT CCT Tyr Ser Ser Ser Asn Met Leu Ile Asn Thr Asn Val Glu Ser Leu Pro 430 435 440	1442
TCT CCA GAG GCC TCA CTG AAG CCA GGT CCA CTT ACC CTG ACT CTG CAA Ser Pro Glu Ala Ser Val Lys Pro Gly Pro Leu Thr Leu Thr Leu Gln 445 450 455	1490
ACC TAC CCA GAT AAC GCC TAC CTG CAG CCT TAT GGG GAC AAG GAG TAC Thr Tyr Pro Asp Asn Ala Tyr Leu Gln Pro Tyr Gly Asp Lys Glu Tyr 460 465 470	1538
CTT GTG GTG AAA TAT CTC CGC CAA CCA ATT TAC CTA GAA GTG AGA ATC Pro Val Val Lys Tyr Leu Arg Gln Pro Ile Tyr Leu Glu Val Arg Ile 475 480 485	1586
CTC AAC AGG ACT GAC CCC AAC ATC AAG CTG GTC TTG GAT GAC TGC TGG Leu Asn Arg Thr Asp Pro Asn Ile Lys Leu Val Leu Asp Asp Cys Trp 490 495 500 505	1634
GCA ACA TCC ACA GAG GAC CCA GCC TCT CTC CCC CAG TGG AAT GTT GTC Ala Thr Ser Thr Glu Asp Pro Ala Ser Leu Pro Gln Trp Asn Val Val 510 515 520 520	1682
ATG GAT GGC TGT GAA TAC AAC CTG GAC AAC CAC AGA ACC ACC TTC CAT Met Asp Gly Cys Glu Tyr Asn Leu Asp Asn His Arg Thr Thr Phe His 525 530 535	1730
CCG GTC GCC TCC TCC GTG ACC TAT CCT AAC CAC CAT CAG AGG TTT GAT Pro Val Gly Ser Ser Val Thr Tyr Pro Asn His His Gln Arg Phe Asp 540 545 550	1778
GTG AAG ACC TTT GCC TTT GTG TCA GGG GCC CAA GGG GTC TCT CAA CTG Val Lys Thr Phe Ala Phe Val Ser Gly Ala Gln Gly Val Ser Gln Leu 555 560 565	1826
GTC TAC TTC CAC TGC ACT GTC TTC ATC TGC AAT CAA CTC TCT CCC ACC Val Tyr Phe His Cys Ser Val Phe Ile Cys Asn Gln Leu Ser Pro Thr 570 575 580 585	1874

- 71 -

TTC TCT CTG TGT TCT GTG ACT TGC CAT GGG CCA TCT AGG AGC CGG CGA Phe Ser Leu Cys Ser Val Thr Cys His Gly Pro Ser Arg Ser Arg Arg 590 595 600	1922
GCT ACA GGG ACC ACT GAG GAA GAG AAA ATG ATA GTG AGT CTC CCG GGC Ala Thr Gly Thr Thr Glu Glu Glu Lys Met Ile Val Ser Leu Pro Gly 605 610 615	1970
CCC ATC CTG CTG TTG TCA GAT GGC TCT TCA CTC AGA GAT GCT GTG AAC Pro Ile Leu Leu Leu Ser Asp Gly Ser Ser Leu Arg Asp Ala Val Asn 620 625 630	2018
TCT AAA GGA TCC AGA ACC AAC GGA TAT GTT GCT TTT AAA ACT ATG GTT Ser Lys Gly Ser Arg Thr Asn Gly Tyr Val Ala Phe Lys Thr Met Val 635 640 645	2066
GCT ATG GTT GCT TCA GCA GGC ATC GTG GCA ACT CTA GGC CTC ATC AGC Ala Met Val Ala Ser Ala Gly Ile Val Ala Thr Leu Gly Leu Ile Ser 650 655 660 665	2114
TAC CTG CAC AAA AAA AGA ATC ATG ATG TTA AAT CAC TAATTTGGAT Tyr Leu His Lys Arg Ile Met Met Leu Asn His 670 675	2160
TTTCAATAAA AAGTGGAAAGT AAGCCCTCTTC TAAAAAAA AAAAACCGGA ATTTC	2214

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 713 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg His Arg Gly Asp Ser Gly Arg Pro Leu Ser Trp Leu Ser Ala Ser -36 -35 -30 -25
Trp Arg Ser Leu Leu Leu Phe Phe Pro Leu Val Thr Ser Val Asn Ser -20 -20 -15 -10 -5
Ile Gly Val Asn Gln Leu Val Asn Thr Ala Phe Pro Gly Ile Val Thr 1 5 10
Cys His Glu Asn Arg Met Val Val Glu Phe Pro Arg Ile Leu Gly Thr 15 20 25
Lys Ile Glu Tyr Thr Ser Val Val Asp Pro Leu Gly Leu Glu Met Met 30 35 40
Asn Cys Thr Tyr Val Leu Asp Pro Glu Asn Leu Thr Leu Lys Ala Pro 45 50 55 60
Tyr Glu Ala Cys Thr Lys Arg Val Arg Gly His His Gln Met Thr Ile 65 70 75
Arg Leu Ile Asp Asp Asn Ala Ala Leu Arg Gln Glu Ala Leu Met Tyr 80 85 90
His Ile Ser Cys Pro Val Met Gly Ala Glu Gly Pro Asp Gln His Ser 95 100 105
Gly Ser Thr Ile Cys Met Lys Asp Phe Met Ser Phe Thr Phe Asn Phe 110 115 120

- 72 -

Phe Pro Gly Met Ala Asp Glu Asn Val Lys Arg Glu Asp Ser Lys Gln  
125 130 135 140  
Arg Met Gly Trp Ser Leu Val Val Gly Asp Gly Glu Arg Ala Arg Thr  
145 150 155  
Leu Thr Phe Gln Glu Ala Met Thr Gln Gly Tyr Asn Phe Leu Ile Glu  
160 165 170  
Asn Gln Lys Met Asn Ile Gln Val Ser Phe His Ala Thr Gly Val Thr  
175 180 185  
Arg Tyr Ser Gln Gly Asn Ser His Leu Tyr Met Val Pro Leu Lys Leu  
190 195 200  
Lys His Val Ser His Gly Gln Ser Leu Ile Leu Ala Ser Gln Leu Ile  
205 210 215 220  
Cys Val Ala Asp Pro Val Thr Cys Asn Ala Thr His Val Thr Leu Ala  
225 230 235  
Ile Pro Glu Phe Pro Gly Lys Leu Lys Ser Val Asn Leu Gly Ser Gly  
240 245 250  
Asn Ile Ala Val Ser Gln Leu His Lys His Gly Ile Glu Met Glu Thr  
255 260 265  
Thr Asn Gly Leu Arg Leu His Phe Asn Gln Thr Leu Leu Lys Thr Asn  
270 275 280  
Val Ser Glu Lys Cys Leu Pro His Gln Leu Tyr Leu Ser Ser Leu Lys  
285 290 295 300  
Leu Thr Phe His Ser Gln Leu Glu Ala Val Ser Met Val Ile Tyr Pro  
305 310 315  
Glu Cys Leu Cys Glu Ser Thr Val Ser Leu Val Ser Glu Glu Leu Cys  
320 325 330  
Thr Gln Asp Gly Phe Met Asp Val Lys Val His Ser His Gln Thr Lys  
335 340 345  
Pro Ala Leu Asn Leu Asp Thr Leu Arg Val Gly Asp Ser Ser Cys Gln  
350 355 360  
Pro Thr Phe Lys Ala Pro Ala Gln Gly Leu Val Gln Phe Arg Ile Pro  
365 370 375 380  
Leu Asn Gly Cys Gly Thr Arg His Lys Phe Lys Asn Asp Lys Val Ile  
385 390 395  
Tyr Glu Asn Glu Ile His Ala Leu Trp Ala Asp Pro Pro Ser Ala Val  
400 405 410  
Ser Arg Asp Ser Glu Phe Arg Met Thr Val Arg Cys Ser Tyr Ser Ser  
415 420 425  
Ser Asn Met Leu Ile Asn Thr Asn Val Glu Ser Leu Pro Ser Pro Glu  
430 435 440  
Ala Ser Val Lys Pro Gly Pro Leu Thr Leu Thr Leu Gln Thr Tyr Pro  
445 450 455 460  
Asp Asn Ala Tyr Leu Gln Pro Tyr Gly Asp Lys Glu Tyr Pro Val Val  
465 470 475  
Lys Tyr Leu Arg Gln Pro Ile Tyr Leu Glu Val Arg Ile Leu Asn Arg

- 73 -

480	485	490
Thr Asp Pro Asn Ile Lys Leu Val Leu Asp Asp Cys Trp Ala Thr Ser		
495	500	505
Thr Glu Asp Pro Ala Ser Leu Pro Gln Trp Asn Val Val Met Asp Gly		
510	515	520
Cys Glu Tyr Asn Leu Asp Asn His Arg Thr Thr Phe His Pro Val Gly		
525	530	535
Ser Ser Val Thr Tyr Pro Asn His His Gln Arg Phe Asp Val Lys Thr		
545	550	555
Phe Ala Phe Val Ser Gly Ala Gln Gly Val Ser Gln Leu Val Tyr Phe		
560	565	570
His Cys Ser Val Phe Ile Cys Asn Gln Leu Ser Pro Thr Phe Ser Leu		
575	580	585
Cys Ser Val Thr Cys His Gly Pro Ser Arg Ser Arg Arg Ala Thr Gly		
590	595	600
Thr Thr Glu Glu Lys Met Ile Val Ser Leu Pro Gly Pro Ile Leu		
605	610	615
Leu Leu Ser Asp Gly Ser Ser Leu Arg Asp Ala Val Asn Ser Lys Gly		
625	630	635
Ser Arg Thr Asn Gly Tyr Val Ala Phe Lys Thr Met Val Ala Met Val		
640	645	650
Ala Ser Ala Gly Ile Val Ala Thr Leu Gly Leu Ile Ser Tyr Leu His		
655	660	665
Lys Lys Arg Ile Met Met Leu Asn His		
670	675	

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1699 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Sus scrofa
  - (D) DEVELOPMENTAL STAGE: Juvenile
  - (E) HAPLOTYPE: Diploidy
  - (F) TISSUE TYPE: Ovary
  - (G) CELL TYPE: Oocyte
- (ix) FEATURE:
  - (A) NAME/KEY: sig\_peptide
  - (B) LOCATION: 38..445
- (ix) FEATURE:
  - (A) NAME/KEY: mat\_peptide
  - (B) LOCATION: 446..1648
- (ix) FEATURE:

- 74 -

(A) NAME/KEY: CDS  
 (B) LOCATION: 38..1648

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCCGGG TGGAAAGTACC TGTTCCTCCGC AGGGCCT ATG TGG TTG CGG CCG TCC Met Trp Leu Arg Pro Ser -136-135	55
ATC TGG CTC TGC TTT CCG CTG TGT CTT GCT CTG CCA GGC CAG TCT CAG Ile Trp Leu Cys Phe Pro Leu Cys Leu Ala Leu Pro Gly Gln Ser Gln -130 -125 -120 -115	103
CCC AAA GCA GCA GAT GAC CTT GGT GGC CTC TAC TGT GGG CCA AGC AGC Pro Lys Ala Ala Asp Asp Leu Gly Leu Tyr Cys Gly Pro Ser Ser -110 -105 -100	151
TTC CAT TTC TCC ATA AAT CTT CTC AGC CAG GAC ACA GCA ACT CCT CCT Phe His Phe Ser Ile Asn Leu Leu Ser Gln Asp Thr Ala Thr Pro Pro -95 -90 -85	199
GCA CTG GTG GTT TGG GAC AGG CGC GGG CGG CTG CAC AAG CTG CAG AAT Ala Leu Val Val Trp Asp Arg Arg Gly Arg Leu His Lys Leu Gln Asn -80 -75 -70	247
GAC TCT GGC TGT GGC ACG TGG GTC CAC AAG GGC CCA CGG AGC TCC ATG Asp Ser Gly Cys Gly Thr Trp Val His Lys Gly Pro Gly Ser Ser Met -65 -60 -55	295
GGA GTG GAA GCA TCC TAC AGA GGC TGC TAT GTG ACT GAG TGG GAC TCT Gly Val Glu Ala Ser Tyr Arg Gly Cys Tyr Val Thr Glu Trp Asp Ser -50 -45 -40 -35	343
CAC TAC CTC ATG CCC ATT GGA CTT GAA GAA GCA GAT GCA GGT GGA CAC His Tyr Leu Met Pro Ile Gly Leu Glu Ala Asp Ala Gly His -30 -25 -20	391
AGA ACA GTC ACA CAG ACG AAA CTG TTT AAG TGC CCT GTG GAT TTC CTA Arg Thr Val Thr Glu Thr Lys Leu Phe Lys Cys Pro Val Asp Phe Leu -15 -10 -5	439
GCT CTT GAT GTT CCA ACC ATT GGC CTT TGT GAT GCT GTC CCA GTG TGG Ala Leu Asp Val Pro Thr Ile Gly Leu Cys Asp Ala Val Pro Val Trp 1 5 10	487
GAC CGA TTG CCA TGT GCT CCT CCA CCC ATC ACT CAA GGA GAA TGC AAC Asp Arg Leu Pro Cys Ala Pro Pro Ile Thr Gln Gly Glu Cys Lys 15 20 25 30	535
CAG CTT GCC TGC TAC AAC TCG GAA GAG GTC CCT TCT TGT TAC TAT Gln Leu Gly Cys Cys Tyr Asn Ser Glu Glu Val Pro Ser Cys Tyr Tyr 35 40 45	583
GGA AAC ACA GTG ACC TCA CGC TGT ACC CAA GAT GGC CAC TTC TCC ATC Gly Asn Thr Val Thr Ser Arg Cys Thr Gln Asp Gly His Phe Ser Ile 50 55 60	631
GCT GTG TCT CGC AAT GTG ACC TCA CCT CCA CTG CTC TGG GAT TCT GTG Ala Val Ser Arg Asn Val Thr Ser Pro Pro Leu Leu Trp Asp Ser Val 65 70 75	679
CAC CTG GCC TTC AGA AAT GAC AGT GAA TGT AAA CCT GTG ATG GAA ACA His Leu Ala Phe Arg Asn Asp Ser Glu Cys Lys Pro Val Met Glu Thr 80 85 90	727
CAC ACT TTT GTC CTC TTC CGG TTT CCA TTT AGT TCC TGT GGG ACT GCA	775

- 75 -

His Thr Phe Val Leu Phe Arg Phe Pro Phe Ser Ser Cys Gly Thr Ala		
95 . . . . . 100 . . . . . 105 . . . . . 110		
AAA CGG GTA ACT GGG AAC CAG GCG GTA TAT GAA AAT GAG CTG GTA GCA	823	
Lys Arg Val Thr Gly Asn Gln Ala Val Tyr Glu Asn Glu Leu Val Ala		
115 . . . . . 120 . . . . . 125		
GCT CGG GAT GTG AGG ACT TGG AGC CAT GGT TCT ATT ACC CGA GAC AGC	871	
Ala Arg Asp Val Arg Thr Trp Ser His Gly Ser Ile Thr Arg Asp Ser		
130 . . . . . 135 . . . . . 140		
ATC TTC AGG CTT CGA GTC AGT TGT ATC TAC TCT GTA AGT AGC AGT GCT	919	
Ile Phe Arg Leu Arg Val Ser Cys Ile Tyr Ser Val Ser Ser Ala		
145 . . . . . 150 . . . . . 155		
CTC CCA GTT AAC ATC CAG GTT TTC ACT CTC CCA CCA CCG CTT CCG GAG	967	
Leu Pro Val Asn Ile Gln Val Phe Thr Leu Pro Pro Pro Leu Pro Glu		
160 . . . . . 165 . . . . . 170		
ACC CAC CCT GGA CCT CTT ACT CTG GAG CTT CAG ATT GCC AAA GAT GAA	1015	
Thr His Pro Gly Pro Leu Thr Leu Glu Leu Gln Ile Ala Lys Asp Glu		
175 . . . . . 180 . . . . . 185 . . . . . 190		
CCG TAT GGC TCC TAC TAC AAT GCT AGT GAC TAC CCG GTG GTG AAA TTG	1063	
Arg Tyr Gly Ser Tyr Tyr Asn Ala Ser Asp Tyr Pro Val Val Lys Leu		
195 . . . . . 200 . . . . . 205		
CTT CGG GAG CCC ATC TAT GTG GAG GTC TCT ATC CCT CAC CGA ACA GAC	1111	
Leu Arg Glu Pro Ile Tyr Val Glu Val Ser Ile Arg His Arg Thr Asp		
210 . . . . . 215 . . . . . 220		
CCC AGT CTC GGG CTG CAC CTG CAC CAG TGC TGG GCC ACA CCC GGG ATG	1159	
Pro Ser Leu Gly Leu His Leu His Gln Cys Trp Ala Thr Pro Gly Met		
225 . . . . . 230 . . . . . 235		
AGC CCC CTG CTC CAG CCA CAG TGG CCC ATG CTA GTC AAT GGA TGC CCC	1207	
Ser Pro Leu Leu Gln Pro Gln Trp Pro Met Leu Val Asn Gly Cys Pro		
240 . . . . . 245 . . . . . 250		
TAC ACT CGA GAC AAC TAC CAG ACC AAA CTG ATC CCT GTC CAG AAA GCC	1255	
Tyr Thr Gly Asp Asn Tyr Gln Thr Lys Leu Ile Pro Val Gln Lys Ala		
255 . . . . . 260 . . . . . 265 . . . . . 270		
TCA AAC CTG CTA TTT CCT TCT CAC TAC CAG CCG CCT TTC AGT GTT TCC ACC	1303	
Ser Asn Leu Leu Phe Pro Ser His Tyr Gln Arg Phe Ser Val Ser Thr		
275 . . . . . 280 . . . . . 285		
TTC AGT TTT GTG GAC TCT GTG GCA AAG CAG GCA CTC AAG GGA CCG GTG	1351	
Phe Ser Phe Val Asp Ser Val Ala Lys Gln Ala Leu Lys Gly Pro Val		
290 . . . . . 295 . . . . . 300		
TAT CTG CAT TGT ACT GCA TCG GTC TGC AAG CCT GCA GGG GCA CCC ATC	1399	
Tyr Leu His Cys Thr Ala Ser Val Cys Lys Pro Ala Gly Ala Pro Ile		
305 . . . . . 310 . . . . . 315		
TGT GTG ACA ACC TGT CCT GCT GCC AGA CGA AGA AGA AGT TCT GAC ATC	1447	
Cys Val Thr Thr Cys Pro Ala Ala Arg Arg Arg Ser Ser Asp Ile		
320 . . . . . 325 . . . . . 330		
CAT TTT CAG AAT GGC ACT GCT AGC ATT TCT AGC AAG GGT CCC ATG ATT	1495	
His Phe Gln Asn Gly Thr Ala Ser Ile Ser Ser Lys Gly Pro Met Ile		
335 . . . . . 340 . . . . . 345 . . . . . 350		
CTA CTC CAA GCC ACT CGG GAC TCT TCA GAA AGG CTC CAT AAA TAC TCA	1543	
Leu Leu Gln Ala Thr Arg Asp Ser Ser Glu Arg Leu His Lys Tyr Ser		
355 . . . . . 360 . . . . . 365		

- 76 -

AGG CCT CCT GTC GAC TCC CAT GCT CTG TGG GTG GCT GGC CTC TTG GGA Arg Pro Pro Val Asp Ser His Ala Leu Trp Val Ala Gly Leu Leu Gly 370 375 380	1591
AGC TTA ATT ATT GCA GCC TTG TTA GTG TCC TAC CTG GTC TTC AGG AAA Ser Leu Ile Ile Gly Ala Leu Val Ser Tyr Leu Val Phe Arg Lys 385 390 395	1639
TGG AGA TGAGTTACTC AGACCAAATG TGTCAATAAA ACCAATAAAA CAAACCGGA Trp ARG 400	1695
ATTC	1699

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 536 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Trp Leu Arg Pro Ser Ile Trp Leu Cys Phe Pro Leu Cys Leu Ala -136 -135 -130 -125
Leu Pro Gly Gln Ser Gln Pro Lys Ala Ala Asp Asp Leu Gly Gly Leu -120 -115 -110 -105
Tyr Cys Gly Pro Ser Ser Phe His Phe Ser Ile Asn Leu Leu Ser Gln -100 -95 -90
Asp Thr Ala Thr Pro Pro Ala Leu Val Val Trp Asp Arg Arg Gly Arg -85 -80 -75
Leu His Lys Leu Gln Asn Asp Ser Gly Cys Gly Thr Trp Val His Lys -70 -65 -60
Gly Pro Gly Ser Ser Met Gly Val Glu Ala Ser Tyr Arg Gly Cys Tyr -55 -50 -45
Val Thr Glu Trp Asp Ser His Tyr Leu Met Pro Ile Gly Leu Glu Glu -40 -35 -30 -25
Ala Asp Ala Gly Gly His Arg Thr Val Thr Glu Thr Lys Leu Phe Lys -20 -15 -10
Cys Pro Val Asp Phe Leu Ala Leu Asp Val Pro Thr Ile Gly Leu Cys -5 1 5
Asp Ala Val Pro Val Trp Asp Arg Leu Pro Cys Ala Pro Pro Pro Ile 10 15 20
Thr Gln Gly Glu Cys Lys Gln Leu Gly Cys Cys Tyr Asn Ser Glu Glu 25 30 35 40
Val Pro Ser Cys Tyr Tyr Gly Asn Thr Val Thr Ser Arg Cys Thr Gln 45 50 55
Asp Gly His Phe Ser Ile Ala Val Ser Arg Asn Val Thr Ser Pro Pro 60 65 70
Leu Leu Trp Asp Ser Val His Leu Ala Phe Arg Asn Asp Ser Glu Cys

- 77 -

75	80	85
Lys Pro Val Met Glu Thr His Thr Phe Val Leu Phe Arg Phe Pro Phe		
90	95	100
Ser Ser Cys Gly Thr Ala Lys Arg Val Thr Gly Asn Gln Ala Val Tyr		
105	110	115
Glu Asn Glu Leu Val Ala Ala Arg Asp Val Arg Thr Trp Ser His Gly		
125	130	135
Ser Ile Thr Arg Asp Ser Ile Phe Arg Leu Arg Val Ser Cys Ile Tyr		
140	145	150
Ser Val Ser Ser Ala Leu Pro Val Asn Ile Gln Val Phe Thr Leu		
155	160	165
Pro Pro Pro Leu Pro Glu Thr His Pro Gly Pro Leu Thr Leu Glu Leu		
170	175	180
Gln Ile Ala Lys Asp Glu Arg Tyr Gly Ser Tyr Tyr Asn Ala Ser Asp		
185	190	195
Tyr Pro Val Val Lys Leu Leu Arg Glu Pro Ile Tyr Val Glu Val Ser		
205	210	215
Ile Arg His Arg Thr Asp Pro Ser Leu Gly Leu His Leu His Gln Cys		
220	225	230
Trp Ala Thr Pro Gly Met Ser Pro Leu Leu Gln Pro Gln Trp Pro Met		
235	240	245
Leu Val Asn Gly Cys Pro Tyr Thr Gly Asp Asn Tyr Gln Thr Lys Leu		
250	255	260
Ile Pro Val Gln Lys Ala Ser Asn Leu Leu Phe Pro Ser His Tyr Gln		
265	270	275
Arg Phe Ser Val Ser Thr Phe Ser Phe Val Asp Ser Val Ala Lys Gln		
285	290	295
Ala Leu Lys Gly Pro Val Tyr Leu His Cys Thr Ala Ser Val Cys Lys		
300	305	310
Pro Ala Gly Ala Pro Ile Cys Val Thr Thr Cys Pro Ala Ala Arg Arg		
315	320	325
Arg Arg Ser Ser Asp Ile His Phe Gln Asn Gly Thr Ala Ser Ile Ser		
330	335	340
Ser Lys Gly Pro Met Ile Leu Leu Gln Ala Thr Arg Asp Ser Ser Glu		
345	350	355
Arg Leu His Lys Tyr Ser Arg Pro Pro Val Asp Ser His Ala Leu Trp		
365	370	375
Val Ala Gly Leu Leu Gly Ser Leu Ile Ile Gly Ala Leu Leu Val Ser		
380	385	390
Tyr Leu Val Phe Arg Lys Trp Arg		
395	400	

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1326 base pairs

- 78 -

(B) TYPE: nucleic acid	51
(C) STRANDEDNESS: double	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: cDNA	
(iii) HYPOTHETICAL: NO	
(iv) ANTI-SENSE: NO	
(vi) ORIGINAL SOURCE:	
(A) ORGANISM: <i>Sus scrofa</i>	
(D) DEVELOPMENTAL STAGE: Juvenile	
(E) HAPLOTYPE: Diploid	
(F) TISSUE TYPE: Ovary	
(G) CELL TYPE: Oocyte	
(ix) FEATURE:	
(A) NAME/KEY: sig_peptide	
(B) LOCATION: 25..105	
(ix) FEATURE:	
(A) NAME/KEY: mat_peptide	
(B) LOCATION: 106..1290	
(ix) FEATURE:	
(A) NAME/KEY: CDS	
(B) LOCATION: 25..1290	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:	
TTCCGGG GCCTTGTGAG TGCC ATG GCG CCG AGC TGG AGG TTC TTC GTC Met Ala Pro Ser Trp Arg Phe Phe Val	51
-27 -25 -20	
TTT CTG CTC TGG GGA GGT ACA GAG CTA TGC AGC CCG CAG CCC GTC Phe Leu Leu Trp Gly Thr Glu Leu Cys Ser Pro Gln Pro Val	99
-15 -10 -5	
CAG GAC GAA GGC CAG CGC TTG AGG CCC TCA AAG CCA CCC ACC GTA Gln Asp Glu Gly Gln Arg Leu Arg Pro Ser Lys Pro Pro Thr Val	147
1 5 10	
GTC GAG TGT CAG GAG GCC CAG CTG GTG GTC ATT GTC AGC AAA GAC Val Glu Cys Gln Glu Ala Gln Leu Val Ile Val Ser Lys Asp	195
20 25 30	
TTC GGT ACC GGG AAG CTC ATC AGG CCT GCA GAT CTC AGC CTG GGC Phe Gly Thr Gly Lys Leu Ile Arg Pro Ala Asp Leu Ser Leu Gly	243
35 40 45	
GCA AAG TGT GAG CCG CTG GTC TCT CAG GAC ACG GAC GCA GTG GTC Ala Lys Cys Glu Pro Leu Val Ser Gln Asp Thr Asp Ala Val Val	291
55 60	
TTC GAG GTT GGG CTG CAC GAG TGT GGC AGC AGC TTG CAG GTG ACT Phe Glu Val Gly Leu His Cys Gly Ser Ser Leu Gin Val Thr	339
65 70 75	
GAT GCT CTG GTG TAC AGC ACC TTC CTG CGC CAT GAC CCC CGC CCT Asp Ala Leu Val Tyr Ser Thr Phe Leu Arg His Asp Pro Arg Pro	387
80 85 90	
GGA AAC CTG TCC ATC CTG AGG ACG AAC CGT GGC GAG GTC CCC ATC Gly Asn Leu Ser Ile Leu Arg Thr Asn Arg Ala Glu Val Pro Ile	435
100 105 110	

- 79 -

GAG TGT CAC TAC CCC AGG CAG GGC AAC GTG AGC AGC TGG GCC ATC CTG Glu Cys His Tyr Pro Arg Gln Gly Asn Val Ser Ser Trp Ala Ile Leu 115	120	125	483
CCC ACC TGG GTG CCC TTC AGG ACC ACG GTG TTC TCC GAG GAG AAG CTG Pro Thr Trp Val Pro Phe Arg Thr Thr Val Phe Ser Glu Lys Leu 130	135	140	531
GTC TTC TCT CTG CGC CTG ATG GAG GAA AAC TGG AGT GCC GAG AAG ATG Val Phe Ser Leu Arg Leu Met Glu Glu Asn Trp Ser Ala Glu Lys Met 145	150	155	579
ACG CCC ACC TTC CAG CTG GGG GAC AGA GCC CAC CTC CAG GCC CAA GTC Thr Pro Thr Phe Gln Leu Gly Asp Arg Ala His Leu Gln Ala Gln Val 160	165	170	627
CAC ACC GGC AGC CAC CTG CCA CTG AGG CTG TTT CTG GAC CAC TGT GTG His Thr Gly Ser His Val Pro Leu Arg Leu Phe Val Asp His Cys Val 175	180	185	675
GCC ACG CTG ACG CGG GAC TGG AAC ACC TCC CCC TCT CAC ACC ATC GTG Ala Thr Leu Thr Pro Asp Trp Asn Thr Ser Pro Ser His Thr Ile Val 195	200	205	723
GAC TTC CAC GGC TGT CTC AGT GAC TGG GGT CTC ACT GAG GCC TCA TCT GCT Asp Phe His Gly Cys Leu Val Asp Gly Leu Thr Glu Ala Ser Ser Ala 210	215	220	771
TTC AAA GCA CCT AGA CCT GGA CCA GAG ACG CTC CAG TTC ACC GTG CAT Phe Lys Ala Pro Arg Pro Gly Pro Glu Thr Leu Gln Phe Thr Val Asp 225	230	235	819
GTG TTC CAT TTT GCT AAT GAT TCC AGA AAC ACG ATC TAC ATC ACC TGC Val Phe His Phe Ala Asn Asp Ser Arg Asn Thr Ile Tyr Ile Thr Cys 240	245	250	867
CAT CTG AAG GTC ACT CCG GCT GAC CGA GTC CCG GAC CAA CTC AAC AAA His Leu Lys Val Thr Pro Ala Asp Arg Val Pro Asp Gln Leu Asn Lys 255	260	265	915
GCC TGT TCC TTC AGC AAG TCC TCC AAC AGG TGG TCC CCG GTG GAA GGG Ala Cys Ser Phe Ser Lys Ser Asn Arg Trp Ser Pro Val Glu Gly 275	280	285	963
CCT GCT GTT ATC TGT CGT TGC TGT CAC AAG GGG CAG TGT GGT ACC CCA Pro Ala Val Ile Cys Arg Cys Cys His Lys Gly Gln Cys Gly Thr Pro 290	295	300	1011
AGC CTT TCC AGG AAG CTG TCT ATG CCG AAG AGA CAG TCT GCT CCC CGC Ser Leu Ser Arg Lys Leu Ser Met Pro Lys Arg Gln Ser Ala Pro Arg 305	310	315	1059
AGT CGC AGG CAC GTG ACA GAT GAA GCA GAT GTC ACA GTG GGG CCT CTG Ser Arg Arg His Val Thr Asp Glu Ala Asp Val Thr Val Gly Pro Leu 320	325	330	1107
ATC TTC CTG GGC AAG ACG ACT GAC CAC GGT GTG GAA GGG TCC ACC TCC Ile Phe Leu Gly Lys Thr Ser Asp His Gly Val Glu Gly Ser Thr Ser 335	340	345	1155
TCC CCC ACC TCG GTG ATG GTG GGC TTG GGC CTG GCC ACC GTG GTG ACC Ser Pro Thr Ser Val Met Val Gly Leu Gly Leu Ala Thr Val Val Thr 355	360	365	1203
TTG ACT CTG GCT ACC ATT GTC CTG GGT GTG CCC AGG AGG CGT CGG GCT Leu Thr Leu Ala Thr Ile Val Leu Gly Val Pro Arg Arg Arg Ala			1251

- 80 -

370

375

380

GCT GCC CAC CTT GTG TCC CCC GTG TCT CCT TCC CAA TAAAAGGAGA  
 Ala Ala His Leu Val Cys Pro Val Ser Ala Ser Gln  
 385 390

1297

AACATGAAAG AAAAAGAAAA CGGAAATTTC

1326

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 421 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Pro Ser Trp Arg Phe Phe Val Cys Phe Leu Leu Trp Gly Gly  
 -27 -25 -20 -15

Thr Glu Leu Cys Ser Pro Gln Pro Val Trp Gln Asp Glu Gly Gln Arg  
 -10 -5 1 5

Leu Arg Pro Ser Lys Pro Pro Thr Val Met Val Glu Cys Gln Glu Ala  
 10 15 20

Gln Leu Val Val Ile Val Ser Lys Asp Leu Phe Gly Thr Gly Lys Leu  
 25 30 35

Ile Arg Pro Ala Asp Leu Ser Leu Gly Pro Ala Lys Cys Glu Pro Leu  
 40 45 50

Val Ser Gln Asp Thr Asp Ala Val Val Arg Phe Glu Val Gly Leu His  
 55 60 65

Glu Cys Gly Ser Ser Leu Gln Val Thr Asp Asp Ala Leu Val Tyr Ser  
 70 75 80 85

Thr Phe Leu Arg His Asp Pro Arg Pro Ala Gly Asn Leu Ser Ile Leu  
 90 95 100

Arg Thr Asn Arg Ala Glu Val Pro Ile Glu Cys His Tyr Pro Arg Gln  
 105 110 115

Gly Asn Val Ser Ser Trp Ala Ile Leu Pro Thr Trp Val Pro Phe Arg  
 120 125 130

Thr Thr Val Phe Ser Glu Glu Lys Leu Val Phe Ser Leu Arg Leu Met  
 135 140 145

Glu Glu Asn Trp Ser Ala Glu Lys Met Thr Pro Thr Phe Gln Leu Gly  
 150 155 160 165

Asp Arg Ala His Leu Gln Ala Gln Val His Thr Gly Ser His Val Pro  
 170 175 180

Leu Arg Leu Phe Val Asp His Cys Val Ala Thr Leu Thr Pro Asp Trp  
 185 190 195

Asn Thr Ser Pro Ser His Thr Ile Val Asp Phe His Gly Cys Leu Val  
 200 205 210

Asp Gly Leu Thr Glu Ala Ser Ser Ala Phe Lys Ala Pro Arg Pro Gly  
 215 220 225

- 81 -

Pro Glu Thr Leu Gln Phe Thr Val Asp Val Phe His Phe Ala Asn Asp  
 230 235 240 245  
 Ser Arg Asn Thr Ile Tyr Ile Thr Cys His Leu Lys Val Thr Pro Ala  
 250 255 260  
 Asp Arg Val Pro Asp Gln Leu Asn Lys Ala Cys Ser Phe Ser Lys Ser  
 265 270 275  
 Ser Asn Arg Trp Ser Pro Val Glu Gly Pro Ala Val Ile Cys Arg Cys  
 280 285 290  
 Cys His Lys Gly Gln Cys Gly Thr Pro Ser Leu Ser Arg Lys Leu Ser  
 295 300 305  
 Met Pro Lys Arg Gln Ser Ala Pro Arg Ser Arg Arg His Val Thr Asp  
 310 315 320 325  
 Glu Ala Asp Val Thr Val Gly Pro Leu Ile Phe Leu Gly Lys Thr Ser  
 330 335 340  
 Asp His Gly Val Glu Gly Ser Thr Ser Ser Pro Thr Ser Val Met Val  
 345 350 355  
 Gly Leu Gly Leu Ala Thr Val Val Thr Leu Thr Leu Ala Thr Ile Val  
 360 365 370  
 Leu Gly Val Pro Arg Arg Arg Ala Ala Ala His Leu Val Cys Pro  
 375 380 385  
 Val Ser Ala Ser Gln  
 390

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1338 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Oryctolagus cuniculus
  - (D) DEVELOPMENTAL STAGE: Juvenile
  - (E) HAPLOTYPE: Diploidy
  - (F) TISSUE TYPE: Ovary
  - (G) CELL TYPE: Oocyte
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 17..1261

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAATTCGGCGG CGGGCC TAC GGG CTC TTC GTT TGC CTA CTG CTC TGG GGA  
 Tyr Gly Leu Phe Val Cys Leu Leu Leu Trp Gly  
 1 5 10

- 82 -

GGC TCG GAG CTG TGC TGC CCC CAG CGG CTC TGG TTC TGG CAG GGC GGG Gly Ser Glu Leu Cys Cys Pro Gln Pro Leu Trp Phe Trp Gln Gly Gly 15 20 25	97
ACC CGC CAG CCC CGG CCC TCC GTG ACG CCC GTG GTG GTG GAG TGT CTG Thr Arg Gln Pro Ala Pro Ser Val Thr Pro Val Val Val Glu Cys Leu 30 35 40	145
GAG GCC CGG CTC GTG GTC ACG GTC AGC AGG GAC CTT TTT GGC ACC CGG Glu Ala Arg Leu Val Val Thr Val Ser Arg Asp Leu Phe Gly Thr Gly 45 50 55	193
AAG CTC ATC CAG GAG GCC GAC CTC AGC CTG GGC CCC GAG GGC TGC GAG Lys Leu Ile Gln Glu Ala Asp Leu Ser Leu Gly Pro Glu Gly Cys Glu 60 65 70 75	241
CCC CAG GCC TCC ACG GAC GCC GTG GTC AGG TTC GAG GTC GGG CTG CAT Pro Gln Ala Ser Thr Asp Ala Val Val Arg Phe Glu Val Gly Leu His 80 85 90	289
GAA TGT GGT AAC AGC GTG CAG GTG ACT GAC GAC TCC CTG GTG TAC AGC Glu Cys Gly Asn Ser Val Gln Val Thr Asp Asp Ser Leu Val Tyr Ser 95 100	337
TCC TTC CTG CTC CAC GAC CCC CGC CCC GCA AAC CTC TCC ATC CTC Ser Phe Leu Leu His Asp Pro Arg Pro Ala Gly Asn Leu Ser Ile Leu 110 115 120	385
AGC AAC AAC CGC GCC GAG GTC CCC ATC GAG TGC CGC TAC CCC AGG CAG Arg Thr Asn Arg Ala Glu Val Pro Ile Glu Cys Arg Tyr Pro Arg Gln 125 130 135	433
GCG AAC GTG AGC AGC CGG CGC ATC CTG CCG ACC TGG GTG CCC TTC TGG Gly Asn Val Ser Ser Arg Ala Ile Leu Pro Thr Trp Val Pro Phe Trp 140 145 150 155	481
ACC ACG GTA CTG TCA GAG GAG AGG CTG CTG TTC TCC TCC CTG CGC CTC ATG Thr Thr Val Leu Ser Glu Glu Arg Leu Val Phe Ser Leu Arg Leu Met 160 165 170	529
GAG GAG AAC TGG AGC CGA GAA AAG ATG TCC CCC ACC TTC CAC CTG CGC Glu Glu Asn Trp Ser Arg Glu Lys Met Ser Pro Thr Phe His Leu Gly 175 180 185	577
GAC ACG GCC CAC CTG CAG GCA GAG GTC CGC ACG GGC AGC CAC CGC CCC Asp Thr Ala His Leu Gln Ala Glu Val Arg Thr Gly Ser His Pro Pro 190 195 200	625
CTG CTG CTG TTC GTG GAT CCC TCC GTG GCC ACC CCG ACA CGG GAC CGG Leu Leu Leu Phe Val Asp Arg Cys Val Ala Thr Pro Thr Arg Asp Gln 205 210 215	673
AGC GGC TCC CCC TAT CAC ACC ATC GTG GAC TTG CAC GGC TGT CTT GTG Ser Gly Ser Pro Tyr His Thr Ile Val Asp Leu His Gly Cys Leu Val 220 225 230 235	721
GAT GGC CTC TCC GAT GGG GCT TCC AAG TTC AAA GCC CCC AGG CGG AAG Asp Gly Leu Ser Asp Gly Ala Ser Lys Phe Lys Ala Pro Arg Pro Lys 240 245 250	769
CCG GAC GTG CTC CAG TTC ATG GTG GCC GTG TTC CAC TTC GCT AAT GAC Pro Asp Val Leu Gln Phe Met Val Ala Val Phe His Phe Ala Asn Asp 255 260 265	817
TCC AGG CAC ACG GTC TAC ATC ACG TGT GTC CTC AGG GTC ATT CCT GCC Ser Arg His Thr Val Tyr Ile Thr Cys His Leu Arg Val Ile Pro Ala 270 275 280	865

- 83 -

CAG CAA GCC CCG GAC CGG CTC AAC AAG GCT TGT TCT TTC AAC CAG TCC Gln Gln Ala Pro Asp Arg Leu Asn Lys Ala Cys Ser Phe Asn Gln Ser 285 290 295	913
TCC AGC AGC TGG CCC CGG GTG GAA GGC AGT GCA GAC ATC TGT GAG TGT Ser Ser Ser Trp Ala Pro Val Glu Gly Ser Ala Asp Ile Cys Glu Cys 300 305 310 315	961
TGC GGC AAC GGT GAC TGT GAC CTC ATC GCA GGC TCC CCC ATG AAC CAG Cys Gly Asn Gly Asp Cys Asp Leu Ile Ala Gly Ser Pro Met Asn Gln 320 325 330 335	1009
AAC CAT GCT GCC CGG TCC TCT CTG CGA AGC CGC AGG CAC GTG ACG GAA Asn His Ala Ala Arg Ser Ser Leu Arg Ser Arg Arg His Val Thr Glu 335 340 345 350	1057
GAA GCA GAC GTC ACC GTG GGC CGG CTG ATC TTC CTG GGG AAG GCT GGT Glu Ala Asp Val Thr Val Gly Pro Leu Ile Phe Leu Gly Lys Ala Gly 350 355 360 365	1105
GAC CCT GCC GGC ACA GAG GGG CTG GCC TCT GCT GCG CAG GCG ACC CTG Asp Pro Ala Gly Thr Glu Gly Leu Ala Ser Ala Ala Gln Ala Thr Leu 365 370 375 380	1153
GTG CTG GCC CTT CGC ATG GGC ACC ATT GTG TTC CTG GCT GTG GCT GCT Val Leu Gly Leu Arg Met Ala Thr Ile Val Phe Leu Ala Val Ala Ala 380 385 390 395	1201
GTG GTC CTG GGC CTC ACC AGG GGG CGC CAC GCT GCT TCC CAC CCC AAG Val Val Leu Gly Leu Thr Arg Gly Arg His Ala Ala Ser His Pro Arg 400 405 410 415	1249
TCT GCT TCC CAA TAAAAAATCA TGACTTCAA AAAAAAAA AAAAAAAA Ser Ala Ser Gln 415	1301
AAAAAAAAAA AAAAAAAA AAACGGCCG CGAACATC	1338

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 415 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Tyr Gly Leu Phe Val Cys Leu Leu Leu Trp Gly Gly Ser Glu Leu Cys 1 5 10 15	15
Cys Pro Gln Pro Leu Trp Phe Trp Gln Gly Gly Thr Arg Gln Pro Ala 20 25 30	30
Pro Ser Val Thr Pro Val Val Val Glu Cys Leu Glu Ala Arg Leu Val 35 40 45	45
Val Thr Val Ser Arg Asp Leu Phe Gly Thr Gly Lys Leu Ile Gln Glu 50 55 60	60
Ala Asp Leu Ser Leu Gly Pro Glu Gly Cys Glu Pro Gln Ala Ser Thr 65 70 75 80	80
Asp Ala Val Val Arg Phe Glu Val Gly Leu His Glu Cys Gly Asn Ser 85 90 95	95

- 84 -

Val	Gln	Val	Thr	Asp	Asp	Ser	Leu	Val	Tyr	Ser	Ser	Phe	Leu	Leu	His
100							105					110			
Asp	Pro	Arg	Pro	Ala	Gly	Asn	Leu	Ser	Ile	Leu	Arg	Thr	Asn	Arg	Ala
115							120				125				
Glu	Val	Pro	Ile	Glu	Cys	Arg	Tyr	Pro	Arg	Gln	Gly	Asn	Val	Ser	Ser
130						135				140					
Arg	Ala	Ile	Leu	Pro	Thr	Trp	Val	Pro	Phe	Trp	Thr	Thr	Val	Leu	Ser
145						150				155			160		
Glu	Glu	Arg	Leu	Val	Phe	Ser	Leu	Arg	Leu	Met	Glu	Glu	Asn	Trp	Ser
165						170				175					
Arg	Glu	Lys	Met	Ser	Pro	Thr	Phe	His	Leu	Gly	Asp	Thr	Ala	His	Leu
180						185				190					
Gln	Ala	Glu	Val	Arg	Thr	Gly	Ser	His	Pro	Pro	Leu	Leu	Phe	Val	
195						200					205				
Asp	Arg	Cys	Val	Ala	Thr	Pro	Thr	Arg	Asp	Gln	Ser	Gly	Ser	Pro	Tyr
210						215				220					
His	Thr	Ile	Val	Asp	Leu	His	Gly	Cys	Leu	Val	Asp	Gly	Leu	Ser	Asp
225						230				235			240		
Gly	Ala	Ser	Lys	Phe	Lys	Ala	Pro	Arg	Pro	Lys	Pro	Asp	Val	Leu	Gln
245						250				255					
Phe	Met	Val	Ala	Val	Phe	His	Phe	Ala	Asn	Asp	Ser	Arg	His	Thr	Val
260						265				270					
Tyr	Ile	Thr	Cys	His	Leu	Arg	Val	Ile	Pro	Ala	Gln	Gln	Ala	Pro	Asp
275						280				285					
Arg	Leu	Asn	Lys	Ala	Cys	Ser	Phe	Asn	Gln	Ser	Ser	Ser	Trp	Ala	
290						295				300					
Pro	Val	Glu	Gly	Ser	Ala	Asp	Ile	Cys	Glu	Cys	Cys	Gly	Asn	Gly	Asp
305						310				315			320		
Cys	Asp	Leu	Ile	Ala	Gly	Ser	Pro	Met	Asn	Gln	Asn	His	Ala	Ala	Arg
325						330				335					
Ser	Ser	Leu	Arg	Ser	Arg	Arg	His	Val	Thr	Glu	Glu	Ala	Asp	Val	Thr
340						345				350					
Val	Gly	Pro	Leu	Ile	Phe	Leu	Gly	Lys	Ala	Gly	Asp	Pro	Ala	Gly	Thr
355						360				365					
Glu	Gly	Leu	Ala	Ser	Ala	Ala	Gln	Ala	Thr	Leu	Val	Leu	Gly	Leu	Arg
370						375				380					
Met	Ala	Thr	Ile	Val	Phe	Leu	Ala	Val	Ala	Ala	Val	Val	Leu	Gly	Leu
385						390				395			400		
Thr	Arg	Gly	Arg	His	Ala	Ala	Ser	His	Pro	Arg	Ser	Ala	Ser	Gln	
405										410			415		

(2) INFORMATION FOR SEQ ID NO:9:

- (1) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2381 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

- 85 -

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Canis familiaris*
- (D) DEVELOPMENTAL STAGE: Juvenile
- (E) HAPLOTYPE: Diploid
- (F) TISSUE TYPE: Ovary
- (G) CELL TYPE: Oocyte

### (ix) FEATURES:

(A) NAME/KEY: CDS  
(B) LOCATION: 206..2353

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGATTCGGGG AGCCCTGAAG GAAAGCCTGAA GAACCCCTGCC CGCACCTCCCG CGACCTCAAG	60
ATGTCCTACTC CACTGGAAGA CGGAGAACATC TGGATTGACC CCAACCAAGG ATGCACCTGT	120
ATGCCATCAA GGTTTCTGC AACATGGAGA CAGGTGAGAC CTGGCTATAC CCACCTACCT	180
GGCTGATTTC GTGGTACCGT TGGCC ATG GCA TGC AAA CAG AAA GCA GAC AGT Met Ala Cys Lys Gln Lys Gly Asp Ser	232
1 5	
GGG AGT CCC TCA AGC AGG TTT AGT GCA GAT TGG AGC ACC TAC AGG TCA Gly Ser Pro Ser Ser Arg Phe Ser Ala Asp Trp Ser Thr Tyr Arg Ser	280
10 15 20 25	
CTT TCT TTA TTC TTC ATC CTT GTG ACT TCA GTG AAC TCA CTA GGT GTT Leu Ser Leu Phe Phe Ile Leu Val Thr Ser Val Asn Ser Val Gly Val	328
30 35 40	
ATG CAG TTG GTG AAT CCC ATC TTC CCA GGT ACT GTC ATT TGC CAT GAA Met Gln Leu Val Asn Pro Ile Phe Pro Gly Thr Val Ile Cys His Glu	376
45 50 55	
ATAT AAA ATG ACA GTG GAA TTT CCA AGG GAT CTT GCC ACC AAA AAA TGG Asn Lys Met Thr Val Glu Phe Pro Arg Asp Leu Gly Thr Lys Lys Trp	424
60 65 70	
CAT GCA TCT GTG GTG GAT CCA TTT AGT TTT GAA TTG TTG AAC TGT ACT His Ala Ser Val Val Asp Pro Phe Ser Phe Glu Leu Asn Cys Thr	472
75 80 85	
TCT ATC CTG GAC CCA GAA AGG CTC ACC CTG AAG GCC CCA TAT GAG ACC Ser Ile Leu Asp Pro Glu Lys Leu Thr Leu Lys Ala Pro Tyr Glu Thr	520
90 95 100 105	
TGT AGC AGG AGA GTG CTT GGC CAG CAT CAG ATG GCC ATC AGA CTC ACG Cys Ser Arg Arg Val Leu Gly Gln His Gln Met Ala Ile Arg Leu Thr	568
110 115 120	
GAC AAC AAT GCT GCT TCA AGA CAT ARG GCT TTC ATG TAT CAG ATC AGC Asp Asn Asn Ala Ala Ser Arg His Lys Ala Phe Met Tyr Gln Ile Ser	616
125 130 135	
TGT CCA CTT ATG CAA ACA GAA GAA ACC CAT GAG CAT GCA GGA TCC ACA Cys Pro Val Met Gln Thr Glu Glu Thr His Glu His Ala Gly Ser Thr	664
140 145 150	
ATC TGC ACA AAA GAT TCC ATG TCT TTT ACC TTT AAC ATT ATT CCT GGC	712

- 86 -

Ile Cys Thr Lys Asp Ser Met Ser Phe Thr Phe Asn Ile Ile Pro Gly 155 160 165	
ATG GCT GAT GAA AAT ACG AAT CCC AGT GGT GGG AAA TGG ATG ATG GAG Met Ala Asp Glu Asn Thr Asn Pro Ser Gly Gly Lys Trp Met Met Glu 170 175 180 185	760
GTT GAT GAT GCA AAA GCT CAA AAT CTG ACT CTT CGG GAG GCC TTG ATG Val Asp Asp Ala Lys Ala Gln Asn Leu Thr Leu Arg Glu Ala Leu Met 190 195 200	808
CRA GGA TAT AAT TTC CTG TTT GAT AGC CAC AGG CTC AGT GTC CAA GTG Gln Gly Tyr Asn Phe Leu Phe Asp Ser His Arg Leu Ser Val Gln Val 205 210 215	856
TCA TTC AAT GCC ACT GGA GTC ACT CAC TAC ATG CAA GGT AAC AGT CAC Ser Phe Asn Ala Thr Gly Val Thr His Tyr Met Gln Gly Asn Ser His 220 225 230	904
CTC TAC ACA GTG CCT CTG AAC CTT ATA CAC ACA TCT CCT GGG CAG AAG Leu Tyr Thr Val Pro Leu Lys Leu Ile His Thr Ser Pro Gly Gln Lys 235 240 245	952
ATC ATC TTA ACA ACA CGA GTA CTT TGT ATG TCA GAT CCC GTG ACC TGT Ile Ile Leu Thr Thr Arg Val Leu Cys Met Ser Asp Pro Val Thr Cys 250 255 260 265	1000
AAC GCC ACR CRC ATG ACC CTC ACC ATA CCA GAG TTT CCT GGG AAA CTA Asn Ala Thr His Met Thr Leu Thr Ile Pro Glu Phe Pro Gly Lys Leu 270 275 280	1048
CAG TCT GTG AGA TTT GAA AAC ACG AAC TTT CGT GTA AGC CAG CTG CAC Gin Ser Val Arg Phe Glu Asn Thr Asn Phe Arg Val Ser Gln Leu His 285 290 295	1096
AAC CAT GGG ATT GAT AAA GAA GAA TTA AAC GGC TTG AGG TTA CAC TTC Asn His Gly Ile Asp Lys Glu Glu Leu Asn Gly Leu Arg Leu His Phe 300 305 310	1144
AGC AAA TCT CTT CTC AAA ATG AAC TCC TCT GAA AAA TGC CTA CTC TAT Ser Lys Ser Leu Leu Lys Met Asn Ser Ser Glu Lys Cys Leu Leu Tyr 315 320 325	1192
CAG TTC TAC TTA GCA TCT CTC AAG CTG ACC TTT GCC TTT GAA CGG GAC Gln Phe Tyr Leu Ala Ser Leu Lys Leu Thr Phe Ala Phe Glu Arg Asp 330 335 340 345	1240
ACG GTT TCC ACA CTG GTT TAT CCT GAG TGT GTT TGT GAG CCA CCA GTT Thr Val Ser Thr Val Val Tyr Pro Glu Cys Val Cys Glu Pro Pro Val	1288
ACT ATA GTT ACA GGT GAC CTG TGT ACC CAG GAT GGG TTT ATG GAT GTC Thr Ile Val Thr Gly Asp Leu Cys Thr Gln Asp Gly Phe Met Asp Val	1336
AAG GTC TAC AGC CAC CAA ACA AAA CCA GCT CTA AAC TTG GAT ACC CTC Lys Val Tyr Ser His Gln Thr Lys Pro Ala Leu Asn Leu Asp Thr Leu 365 370 375	1384
AGA GTG GGA GAC TCC TCC TGC CAA CCT ACT TTC AAG GCT CCA TCA CAA Arg Val Gly Asp Ser Ser Cys Gln Pro Thr Phe Lys Ala Pro Ser Gln 395 400 405	1432
GGG TTG ACA CTG TTT CAC ATC CCC CTA AAT GGA TGT GGA ACA AGA CTT Gly Leu Thr Leu Phe His Ile Pro Leu Asn Gly Cys Gly Thr Arg Leu 410 415 420 425	1480

- 87 -

AAG TTC AAA GGT GAC ACA GTC ATC TAT GAA AAT GAA ATA CAT GCT CTC Lys Phe Lys Gly Asp Thr Val Ile Tyr Glu Asn Glu Ile His Ala Leu 430 435 440	1528
TCG ACA GAT CTC CCT CCA AGC ACA ATT TCC AGA GAT AGT GAA TTC AGA Trp Thr Asp Leu Pro Pro Ser Thr Ile Ser Arg Asp Ser Glu Phe Arg 445 450 455	1576
ATG ACT GTG AAG TGC CAT TAC AGC AGA GAT GAC CTG CTG ATA AAT ACC Met Thr Val Lys Cys His Tyr Ser Arg Asp Asp Leu Ile Asn Thr 460 465 470	1624
AAT GTC CAA AGT CTT CCT CCC GTG GCC TCA GTG AGG CCT GGT CCA Asn Val Gln Ser Leu Pro Pro Val Ala Ser Val Arg Pro Gly Pro 475 480 485	1672
CTT GCC TTA ATC CTG CAA ACC TAC CCA GAT AAA TCC TAT TTG CGA CCC Leu Ala Leu Ile Leu Gln Thr Tyr Pro Asp Lys Ser Tyr Leu Arg Pro 490 495 500 505	1720
TAT GGG GAT AAG GAG TAT CCT GTG GTG AGA TAC CTC CGC CAA CCA ATT Tyr Gly Asp Lys Glu Tyr Pro Val Val Arg Tyr Leu Arg Gln Pro Ile 510 515 520	1768
TAC CTG CAA GTG AAA GTC CTA AAT AGC GCT GAC CCC AAC ATC AAG CTG Tyr Leu Glu Val Lys Val Leu Asn Arg Ala Asp Pro Asn Ile Lys Leu 525 530 535	1816
GTC TTA GAT GAT TGC TGG GCA ACA CCC ACC ATG GAC CCA GCC TCA CTC Val Leu Asp Asp Cys Trp Ala Thr Pro Thr Met Asp Pro Ala Ser Leu 540 545 550	1864
CCC CAG TGG AAT ATT GTC ATG GAT GCC TGT GAA TAC ATT CTG GAC AAC Pro Gln Trp Asn Ile Val Met Asp Gly Cys Glu Tyr Asn Leu Asp Asn 555 560 565	1912
TAC AGA ACG ACC TTC CAT CCA GTT GCC TCC TCT GTG ACC TAC CCT ACT Tyr Arg Thr Thr Phe His Pro Val Gly Ser Ser Val Thr Tyr Pro Thr 570 575 580 585	1960
CAC TAT CAG AGG TTT GAT GTG AAG ACC TTT GCC TTT ATA TCA GAG GCC His Tyr Gln Arg Phe Asp Val Lys Thr Phe Ala Phe Ile Ser Glu Ala 590 595 600	2008
CAA CTG CTT TCT AGC CTG GTC TAC TTC CAC TGC ACC GCA TTA ATC TGC Gln Val Leu Ser Ser Leu Val Tyr Phe His Cys Thr Ala Leu Ile Cys 605 610 615	2056
AAT CGA CTG TCT CCT GAC TCC CCT CTG TGT TCT GTG ACT TGC CCT GTA Asn Arg Leu Ser Pro Asp Ser Pro Leu Cys Ser Val Thr Cys Pro Val 620 625 630	2104
TCA TCC AGG CAC AGG CGA GCC ACA GGC ACT ACT GAA GAA GAG ARG ATG Ser Ser Arg His Arg Arg Ala Thr Gly Ser Thr Glu Glu Glu Lys Met 635 640 645	2152
ATA GTA AGT CTC CCG GGA CCC ATC CTC CTG TTG GCA GAC AGC TCT TCA Ile Val Ser Leu Pro Gly Pro Ile Leu Leu Ala Asp Ser Ser Ser 650 655 660 665	2200
CTC AGA GAT GGT GTG GAC TCA AAA GGG CAC AGG GCT GCT GGA TAT GTT Leu Arg Asp Gly Val Asp Ser Lys Gly His Arg Ala Ala Gly Tyr Val 670 675 680	2248
GCT TTT AAA ACT GTA GTG GCT GTG GCT GCC TTA GCA GGC CTT GTG GCT Ala Phe Lys Thr Val Val Ala Val Ala Ala Leu Ala Gly Leu Val Ala 685 690 695	2296

- 88 -

GCT CTA GGT CTC ATC ATC TAC CTG CGT AAG AAA AGA ACC ATG GTG TTA Ala Leu Gly Leu Ile Ile Tyr Leu Arg Lys Lys Arg Thr Met Val Leu 700 705 710	2344
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AAT CAC TAAGGATTTC CAAATAAAGT GTCCGGAATT C Asn His 715	2381
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## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 715 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Cys Lys Gln Lys Gly Asp Ser Gly Ser Pro Ser Ser Arg Phe	
1 5 10 15	
Ser Ala Asp Trp Ser Thr Tyr Arg Ser Leu Ser Leu Phe Phe Ile Leu	
20 25 30	
Val Thr Ser Val Asn Ser Val Gly Val Met Gln Leu Val Asn Pro Ile	
35 40 45	
Phe Pro Gly Thr Val Ile Cys His Glu Asn Lys Met Thr Val Glu Phe	
50 55 60	
Pro Arg Asp Leu Gly Thr Lys Lys Trp His Ala Ser Val Val Asp Pro	
65 70 75 80	
Phe Ser Phe Glu Leu Leu Asn Cys Thr Ser Ile Leu Asp Pro Glu Lys	
85 90 95	
Leu Thr Leu Lys Ala Pro Tyr Glu Thr Cys Ser Arg Arg Val Leu Gly	
100 105 110	
Gln His Gln Met Ala Ile Arg Leu Thr Asp Asn Asn Ala Ala Ser Arg	
115 120 125	
His Lys Ala Phe Met Tyr Gln Ile Ser Cys Pro Val Met Gln Thr Glu	
130 135 140	
Glu Thr His Glu His Ala Gly Ser Thr Ile Cys Thr Lys Asp Ser Met	
145 150 155 160	
Ser Phe Thr Phe Asn Ile Ile Pro Gly Met Ala Asp Glu Asn Thr Asn	
165 170 175	
Pro Ser Gly Gly Lys Trp Met Met Glu Val Asp Asp Ala Lys Ala Gln	
180 185 190	
Asn Leu Thr Leu Arg Glu Ala Leu Met Gln Gly Tyr Asn Phe Leu Phe	
195 200 205	
Asp Ser His Arg Leu Ser Val Gln Val Ser Phe Asn Ala Thr Gly Val	
210 215 220	
Thr His Tyr Met Gln Gly Asn Ser His Leu Tyr Thr Val Pro Leu Lys	
225 230 235 240	
Leu Ile His Thr Ser Pro Gly Gln Lys Ile Ile Leu Thr Thr Arg Val	
245 250 255	

- 89 -

Leu Cys Met Ser Asp Pro Val Thr Cys Asn Ala Thr His Met Thr Leu  
260 265 270

Thr Ile Pro Glu Phe Pro Gly Lys Leu Gln Ser Val Arg Phe Glu Asn  
275 280 285

Thr Asn Phe Arg Val Ser Gln Leu His Asn His Gly Ile Asp Lys Glu  
290 295 300

Glu Leu Asn Gly Leu Arg Leu His Phe Ser Lys Ser Leu Leu Lys Met  
305 310 315 320

Asn Ser Ser Glu Lys Cys Leu Leu Tyr Gln Phe Tyr Leu Ala Ser Leu  
325 330 335

Lys Leu Thr Phe Ala Phe Glu Arg Asp Thr Val Ser Thr Val Val Tyr  
340 345 350

Pro Glu Cys Val Cys Glu Pro Pro Val Thr Ile Val Thr Gly Asp Leu  
355 360 365

Cys Thr Gln Asp Gly Phe Met Asp Val Lys Val Tyr Ser His Gln Thr  
370 375 380

Lys Pro Ala Leu Asn Leu Asp Thr Leu Arg Val Gly Asp Ser Ser Cys  
385 390 395 400

Gln Pro Thr Phe Lys Ala Pro Ser Gln Gly Leu Thr Leu Phe His Ile  
405 410 415

Pro Leu Asn Gly Cys Gly Thr Arg Leu Lys Phe Lys Gly Asp Thr Val  
420 425 430

Ile Tyr Glu Asn Ile His Ala Leu Trp Thr Asp Leu Pro Pro Ser  
435 440 445

Thr Ile Ser Arg Asp Ser Glu Phe Arg Met Thr Val Lys Cys His Tyr  
450 455 460

Ser Arg Asp Asp Leu Leu Ile Asn Thr Asn Val Gln Ser Leu Pro Pro  
465 470 475 480

Pro Val Ala Ser Val Arg Pro Gly Pro Leu Ala Leu Ile Leu Gln Thr  
485 490 495

Tyr Pro Asp Lys Ser Tyr Leu Arg Pro Tyr Gly Asp Lys Glu Tyr Pro  
500 505 510

Val Val Arg Tyr Leu Arg Gln Pro Ile Tyr Leu Glu Val Lys Val Leu  
515 520 525

Asn Arg Ala Asp Pro Asn Ile Lys Leu Val Leu Asp Asp Cys Trp Ala  
530 535 540

Thr Pro Thr Met Asp Pro Ala Ser Leu Pro Gln Trp Asn Ile Val Met  
545 550 555 560

Asp Gly Cys Glu Tyr Asn Leu Asp Asn Tyr Arg Thr Thr Phe His Pro  
565 570 575

Val Gly Ser Ser Val Thr Tyr Pro Thr His Tyr Gln Arg Phe Asp Val  
580 585 590

Lys Thr Phe Ala Phe Ile Ser Glu Ala Gln Val Leu Ser Ser Leu Val  
595 600 605

- 90 -

Tyr Phe His Cys Thr Ala Leu Ile Cys Asn Arg Leu Ser Pro Asp Ser  
 610 615 620

Pro Leu Cys Ser Val Thr Cys Pro Val Ser Ser Arg His Arg Arg Ala  
 625 630 635 640

Thr Gly Ser Thr Glu Glu Glu Lys Met Ile Val Ser Leu Pro Gly Pro  
 645 650 655

Ile Leu Leu Ala Asp Ser Ser Ser Leu Arg Asp Gly Val Asp Ser  
 660 665 670

Lys Gly His Arg Ala Ala Gly Tyr Val Ala Phe Lys Thr Val Val Ala  
 675 680 685

Val Ala Ala Leu Ala Gly Leu Val Ala Ala Leu Gly Leu Ile Ile Tyr  
 690 695 700

Leu Arg Lys Lys Arg Thr Met Val Leu Asn His  
 705 710 715

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1325 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Canis familiaris
  - (D) DEVELOPMENTAL STAGE: Juvenile
  - (E) HAPLOTYPE: Diploidy
  - (F) TISSUE TYPE: Ovary
  - (G) CELL TYPE: Oocyte

- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 13..1293

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCCGGG CT ATG GGG CTG AGC TAT GGA ATT TTC ATC TGT TTT CTG	48
Met Gly Leu Ser Tyr Gly Ile Phe Ile Cys Phe Leu	
1                      5                         10	
CTC CTG GGA GGC ATG GAG CTG TGC CCC CAG ACC ATC TGG CCA ACT	96
Leu Leu Gly Gly Met Glu Leu Cys Cys Pro Gln Thr Ile Trp Pro Thr	
15                     20                         25	
GAG ACC TAC TAC CCA TTG ACA TCT AGG CCC CCA GTA ATG GTG GAC TGT	144
Glu Thr Tyr Tyr Pro Leu Thr Ser Arg Pro Pro Val Met Val Asp Cys	
30                     35                         40	
CTG GAG TCC CAG CTG GTG GTC ACT GTC AGC AAA GAC CTT TTT GGT ACT	192
Leu Glu Ser Gln Leu Val Val Thr Val Ser Lys Asp Leu Phe Gly Thr	
45                     50                         55                     60	
GGG AAG CTC ATC AGG CCA GCA GAC CTC ACC CTG GGT CCA GAG AAC TGT	240

- 91 -

Gly Lys Leu Ile Arg Pro Ala Asp Leu Thr Leu Gly Pro Glu Asn Cys		
65	70	75
GAG CCC CTG GTC TCC ATG GAC ACG GAT GAT GTG GTC AGG TTT GAG GTT		288
Glu Pro Leu Val Ser Met Asp Thr Asp Asp Val Val Arg Phe Glu Val		
80	85	90
GGG CTG CAC GAG TGT CGC AGC AGG GTG CRG GTG ACT GAC AAT GCT CTG		336
Gly Leu His Glu Cys Gly Ser Arg Val Gln Val Thr Asp Asn Ala Leu		
95	100	105
GTG TAC AGC ACC TTC CTG ATC CAC AGC CCC CGC CCT GCG GGC AAC CTG		384
Val Tyr Ser Thr Phe Leu Ile His Ser Pro Arg Pro Ala Gly Asn Leu		
110	115	120
TCC ATC CTG AGA ACT ATAT CGT GCC GAG GTT CCC ATC GAG TGC CAC TAC		432
Ser Ile Leu Arg Thr Asn Arg Ala Glu Val Pro Ile Glu Cys His Tyr		
125	130	135
CCC AGG CAC AGC AAT GTG AGC AGC CAG GCC ATC CTG CCC ACT TGG GTG		480
Pro Arg His Ser Asn Val Ser Ser Gln Ala Ile Leu Pro Thr Trp Val		
145	150	155
CCC TTC AGG ACC ACA ATG CTC TTC GAG GAG AAG CTA GTT TTC TCT CTC		528
Pro Phe Arg Thr Thr Met Leu Phe Glu Glu Lys Leu Val Phe Ser Leu		
160	165	170
CGC CTA ATG GAG GAC GAC TGG GCC TCC GAG AAG CAA TCC CCC ACA TTC		576
Arg Leu Met Glu Glu Asp Trp Gly Ser Glu Lys Gln Ser Pro Thr Phe		
175	180	185
CAG CTG GGA GAC ATA GCC CAC CTC CAG GCT GAA GTC CAC ACT GGC AGC		624
Gln Leu Gly Asp Ile Ala His Leu Gln Ala Glu Val His Thr Gly Ser		
190	195	200
CAT ATG CCA CTG CGA CTT TTT GTG GAC CAC TGT GTC GGC ACG CTC ACA		672
His Met Pro Leu Arg Leu Phe Val Asp His Cys Val Ala Thr Leu Thr		
205	210	215
220		
CCA GAT CGG AAT GCC TTC CTT CAT CAC AAA ATT GTG GAC TTC CAT GGC		720
Pro Asp Arg Asn Ala Phe Leu His His Lys Ile Val Asp Phe His Gly		
225	230	235
TGT CTT GTG GAT GGT CTC TAC AAT TCC TCT TCA GCC TTC AAA GCC CCC		768
Cys Leu Val Asp Gly Leu Tyr Asn Ser Ser Ala Phe Lys Ala Pro		
240	245	250
255		
AGA CCC AGG CCA GAG ACT CTT CAG TTC ACA GTG GAT GTT TTC CAC TTT		816
Arg Pro Arg Pro Glu Thr Leu Gln Phe Thr Val Asp Val Phe His Phe		
260	265	
GCT AAG GAC TCA AGA AAC ACG ATC TAT ATC ACC TGC CAT CTG AAG GTC		864
Ala Lys Asp Ser Arg Asn Thr Ile Tyr Ile Thr Cys His Leu Lys Val		
270	275	280
285		
ACT CCG GCT GAC CGA GTC CCA GAC CAG CTA AAC AAA GCT TGT TCC TTC		912
Thr Pro Ala Asp Arg Val Pro Asp Gln Leu Asn Lys Ala Cys Ser Phe		
290	295	300
305		
ATC AAG TCT ACC AAG AGG TGG TAC CCT GTA GAA GGC TCG GCT GAT ATT		960
Ile Lys Ser Thr Lys Arg Trp Tyr Pro Val Glu Gly Ser Ala Asp Ile		
310		
TGT CGC TGT TGT AAC AAA GGC AGC TGT GGC CTT CCA GGC CGG TCC AGG		1008
Cys Arg Cys Cys Asn Lys Gly Ser Cys Gly Leu Pro Gly Ser Arg Ser Arg		
320	325	330

- 92 -

AGG CTG TCC CAC CTA GAG AGA GGG TGG CGC AAG TCT GTT TCC CAC ACT Arg Leu Ser His Leu Glu Arg Gly Trp Arg Lys Ser Val Ser His Thr 335 340 345	1056
AGA AAT CGC AGG CAC GTG ACT GAA GCA GAG ATC ACC GTG GGG CCT Arg Asn Arg Arg His Val Thr Glu Ala Glu Ile Thr Val Gly Pro 350 355 360	1104
CTG ATC TTC CTG GGA AAG CCT AGT GAT CAT GGT ATA GAG GGG TCA ACC Leu Ile Phe Leu Gly Lys Ala Ser Asp His Gly Ile Glu Gly Ser Thr 365 370 375 380	1152
TCT CCT CAC ACC TCT GTG ATG TTG GGC TTA GGC CTG GCC ACG GTG GTA Ser Pro His Thr Ser Val Met Leu Gly Leu Gly Leu Ala Thr Val Val 385 390 395	1200
TCC CTG ACT CTA GCT ACC ATT GTC CTG GTC CTT GCC AAC AGG CAT CGT Ser Leu Thr Leu Ala Thr Ile Val Leu Val Leu Ala Lys Arg His Arg 400 405 410	1248
ACT GCT TCC CAC CCT GTG ATA TGC CCT GCA TCT GTC TCC CAA TAAAGACAATA Thr Ala Ser His Pro Val Ile Cys Pro Ala Ser Val Ser Gln 415 420 425	1300
AGCAAAAGAA ARAAAACCGG AATTC	1325

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 426 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Gly Leu Ser Tyr Gly Ile Phe Ile Cys Phe Leu Leu Gly Gly 1 5 10 15
Met Glu Leu Cys Cys Pro Gln Thr Ile Trp Pro Thr Glu Thr Tyr Tyr 20 25 30
Pro Leu Thr Ser Arg Pro Pro Val Met Val Asp Cys Leu Glu Ser Gln 35 40 45
Leu 'al Val Thr Val Ser Lys Asp Leu Phe Gly Thr Gly Lys Leu Ile 50 55 60
Arg Pro Ala Asp Leu Thr Leu Gly Pro Glu Asn Cys Glu Pro Leu Val 65 70 75 80
Ser Met Asp Thr Asp Asp Val Val Arg Phe Glu Val Gly Leu His Glu 85 90 95
Cys Gly Ser Arg Val Gln Val Thr Asp Asn Ala Leu Val Tyr Ser Thr 100 105 110
Phe Leu Ile His Ser Pro Arg Pro Ala Gly Asn Leu Ser Ile Leu Arg 115 120 125
Thr Asn Arg Ala Glu Val Pro Ile Glu Cys His Tyr Pro Arg His Ser 130 135 140
Asn Val Ser Ser Gln Ala Ile Leu Pro Thr Trp Val Pro Phe Arg Thr 145 150 155 160

- 93 -

Thr	Met	Leu	Phe	Glu	Glu	Lys	Leu	Val	Phe	Ser	Leu	Arg	Leu	Met	Glu
															175
Glu	Asp	Trp	Gly	Ser	Glu	Lys	Gln	Ser	Pro	Thr	Phe	Gln	Leu	Gly	Asp
															190
Ile	Ala	His	Leu	Gln	Ala	Glu	Val	His	Thr	Gly	Ser	His	Met	Pro	Leu
															205
Arg	Leu	Phe	Val	Asp	His	Cys	Val	Ala	Thr	Leu	Thr	Pro	Asp	Arg	Asn
															220
Ala	Phe	Leu	His	His	Lys	Ile	Val	Asp	Phe	His	Gly	Cys	Leu	Val	Asp
															240
Gly	Leu	Tyr	Asn	Ser	Ser	Ser	Ala	Phe	Lys	Ala	Pro	Arg	Pro	Arg	Pro
															255
Glu	Thr	Leu	Gln	Phe	Thr	Val	Asp	Val	Phe	His	Phe	Ala	Lys	Asp	Ser
															270
Arg	Asn	Thr	Ile	Tyr	Ile	Thr	Cys	His	Leu	Lys	Val	Thr	Pro	Ala	Asp
															285
Arg	Val	Pro	Asp	Gln	Leu	Asn	Lys	Ala	Cys	Ser	Phe	Ile	Lys	Ser	Thr
															300
Lys	Arg	Trp	Tyr	Pro	Val	Glu	Gly	Ser	Ala	Asp	Ile	Cys	Arg	Cys	Cys
															320
Asn	Lys	Gly	Ser	Cys	Gly	Leu	Pro	Gly	Arg	Ser	Arg	Arg	Leu	Ser	His
															335
Leu	Glu	Arg	Gly	Trp	Arg	Lys	Ser	Val	Ser	His	Thr	Arg	Asn	Arg	Arg
															340
His	Val	Thr	Glu	Glu	Ala	Glu	Ile	Thr	Val	Gly	Pro	Leu	Ile	Phe	Leu
															355
Gly	Lys	Ala	Ser	Asp	His	Gly	Ile	Glu	Gly	Ser	Thr	Ser	Pro	His	Thr
															370
Ser	Val	Met	Leu	Gly	Leu	Gly	Leu	Ala	Thr	Val	Val	Ser	Leu	Thr	Leu
															385
Ala	Thr	Ile	Val	Leu	Val	Leu	Ala	Lys	Arg	His	Arg	Thr	Ala	Ser	His
															405
Pro	Val	Ile	Cys	Pro	Ala	Ser	Val	Ser	Gln						
															420

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2236 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:

- 94 -

- (A) ORGANISM: *Felis domesticus*
- (D) DEVELOPMENTAL STAGE: Juvenile
- (E) HAPLOTYPE: Diploidy
- (F) TISSUE TYPE: Ovary
- (G) CELL TYPE: Oocyte

(ix) FEATURE:  
      (A) NAME/KEY: CDS  
      (B) LOCATION: 28..2175

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAATTCGCGG CCGCGTACT TTTGGCT ATG GCC TCC AGA CAG AAA GGA GAT Met Ala Ser Arg Gln Lys Gly Asp	51
AGT GGG AGT CCT TCA AGC TGG TTT AAT GCA GAT TGG AGC ACC TAC AGG Ser Gly Ser Pro Ser Ser Trp Phe Asn Ala Asp Trp Ser Thr Tyr Arg	99
10 15 20	
TCA CTT TTT CTA CTC TTT ATC CTC GTG ACT TCA GTG AAT TCC ATA GGT Ser Leu Phe Leu Leu Phe Ile Leu Val Thr Ser Val Asn Ser Ile Gly	147
25 30 35 40	
GTT TTG CAG TTG GTG AAT CCT GTC TTC CCA GGT ACT GTC ACT TGC TAT Val Leu Gln Leu Val Asn Pro Val Phe Pro Gly Thr Val Thr Cys Tyr	195
45 50 55	
GAA ACT AGA ATG GCA GTG GAA TTT CCA AGT GAT TTT GGC ACC AAA AAA Glu Thr Arg Met Ala Val Glu Phe Pro Ser Asp Phe Gly Thr Lys Lys	243
60 65 70	
TGG CAT ACA TCT GTG GTG GAT CCC TTT AGT TTT GAA TTG TTG TTG AAC TGC Trp His Thr Ser Val Val Asp Pro Phe Ser Phe Glu Leu Leu Asn Cys	291
75 80 85	
ACT TAC ATC TTG GAT CCA GAA AAT CTC ACC TTA AAG GCC CCA TAT GAG Thr Tyr Ile Leu Asp Pro Glu Asn Leu Thr Leu Lys Ala Pro Tyr Glu	339
90 95 100	
ACC TGT ACC AGA AGA ACG CTT CGC CAG CAC CGG ATG ATC ATC AGA CTC Thr Cys Thr Arg Arg Thre Leu Gly Gln His Arg Met Ile Ile Arg Leu	387
105 110 115 120	
AGG GAC CAC AAT GCT GCT TCA AGA CAT AAC AGT TTG ATG TAT CAG ATC Lys Asp His Asn Ala Ala Ser Arg His Asn Ser Leu Met Tyr Gln Ile	435
125 130 135	
AAC TGT CCA GTT ATG CAA GCA GAA GAA ACC CAT GAG CAT GCA GGA TCC Asn Cys Pro Val Met Gln Ala Glu Glu Thr His Glu His Ala Gly Ser	483
140 145 150	
ACT ATC TCC ACA AAG GAT TCC ATG TCT TTT ACC TTT AAT GTC ATT CCT Thr Ile Cys Thr Lys Asp Ser Met Ser Phe Thr Phe Asn Val Ile Pro	531
155 160 165	
GGC CTG GCT GAT GAA AAT ACC GAT ATC AGG AAT CGG ATG GGA TGG AGC Gly Leu Ala Asp Glu Asn Thr Asp Ile Lys Asn Pro Met Gly Trp Ser	579
170 175 180	
ATT GAG GTT GGT GAT GGT ACA AAA GCC AAA ACT CTG ACT CTT CAG GAT Ile Val Gly Asp Gly Thr Lys Ala Lys Thr Leu Thr Leu Gln Asp	627
185 190 195 200	
TTC TTG AGA CAR GGA TAC AAT ATC CTG TTT GAT AAC CAC AAC ATC ACC Ala Leu Arg Gln Gly Thr Asn Ile Leu Phe Asp Asn His Lys Ile Thr	675

- 95 -

	205	210	215	
TTC CAG GTG TCA TTC AAT GCC ACT GGA GTG ACT CAC TAC ATG CAA GGT Phe Gln Val Ser Phe Asn Ala Thr Gly Val Thr His Tyr Met Gln Gly	220	225	230	723
AAC AGT CAC CTC TAC ATG GTG CCT CTG AAG TTG ATA CAT GAA TCT CTT Asn Ser His Leu Tyr Met Val Pro Leu Lys Leu Ile His Glu Ser Leu	235	240	245	771
GGG CAG AAG ATC ATC TTA ACA ACA CGA GTG CTT TGT ATG TCA GAT GCT Gly Gln Lys Ile Ile Leu Thr Arg Val Leu Cys Met Ser Asp Ala	250	255	260	819
G TG ACC TGT AAT GCC ACA CAT GTG ACT CTG ACC ATA CCA GAG TTT CCT Val Thr Cys Asn Ala Thr His Val Thr Leu Thr Ile Pro Glu Phe Pro	265	270	275	867
GGG AAG TTA AAA TCT GTG AGC TCT GAA AAT AGG AAC TTT GCT GTA AGC Gly Lys Leu Lys Ser Val Ser Ser Glu Asn Arg Asn Phe Ala Val Ser	285	290	295	915
CAG CTG CAC AAC AAT GGG ATT GAT AAA GAA GAA TCA AGT GGC TTG ACA Gln Leu His Asn Asn Gly Ile Asp Lys Glu Glu Ser Ser Gly Leu Thr	300	305	310	963
TTG CAC TTC AGC AAA ACT CTT CTC AAA ATG GAA TTC TCT GAA AAA TGC Leu His Phe Ser Lys Thr Leu Leu Lys Met Glu Phe Ser Glu Lys Cys	315	320	325	1011
CTA CCC TAT CAG TTC TAC TTA GCT TCA CTC AAG CTG ACC TTT GCC TTT Leu Pro Tyr Gln Phe Tyr Leu Ala Ser Leu Lys Leu Thr Phe Ala Phe	330	335	340	1059
AAT CAA GAG ACT ATA TCC ACG GTG CTT TAT CTC GAG TGT GTC TGT GAG Asn Gln Glu Thr Ile Ser Thr Val Leu Tyr Pro Glu Cys Val Cys Glu	345	350	355	1107
TCA CCA GTT TCT ATA GTT ACA GGT GAC CTG TGT ACT CAG GAT GGG TTT Ser Pro Val Ser Ile Val Thr Gly Asp Leu Cys Thr Gln Asp Gly Phe	365	370	375	1155
ATG GAC ATA AAG GTC TAC AGT CAC CAG ACA AAA CCA GCT CTC AAC TTA Met Asp Ile Lys Val Tyr Ser His Gln Thr Lys Pro Ala Leu Asn Leu	380	385	390	1203
GAA ACC CTA AGG GTG GGA GAC TCA TCC TGC CAA CCT ACC TTC CAG GCT Glu Thr Leu Arg Val Gly Asp Ser Ser Cys Gln Pro Thr Phe Gln Ala	395	400	405	1251
GCA TCT CAA GGG CTG ATA CTG TTT CAC ATA CCC CTG AAT GGA TGC GGG Ala Ser Gln Gly Leu Ile Leu Phe His Ile Pro Leu Asn Gly Cys Gly	410	415	420	1299
ACA AGA CAT AAG TTC AAG GAA GGC AAA GTC ATC TAT GAA AAT GAA ATA Thr Arg His Lys Phe Lys Glu Gly Lys Val Ile Tyr Glu Asn Glu Ile	425	430	435	1347
CAT GCT GTC TGG GCG GAT CTT CCT CCA AGC ACA ATT TCT AGA GAT AGT His Ala Val Trp Ala Asp Leu Pro Pro Ser Thr Ile Ser Arg Asp Ser	445	450	455	1395
GGAA TTC AGA ATG ACA GTG CAG TGC CAT TAC AGC AAA GGT GAC CTG CTA Glu Phe Arg Met Thr Val Gln Cys His Tyr Ser Lys Gly Asp Leu Leu	460	465	470	1443
ATA AAT ACC AGA GTC CAA AGT CTT CCT CCT CTA GAG GCC TCA GTG AGG				1491

- 96 -

Ile Asn Thr Arg Val Gln Ser Leu Pro Pro Leu Glu Ala Ser Val Arg 475 480 485	
CCA GGT CCA CTT GCC TTA ATC CTG CAA ACC TAC CCA GAT AAA TCC TAC Pro Gly Pro Leu Ala Leu Ile Leu Gln Thr Tyr Pro Asp Lys Ser Tyr 490 495 500	1539
CTC CAA CCT TAC GGG GAG AAG GAG TAC CCT GTG GTG AGA TAC CTC CGC Leu Gln Pro Tyr Gly Glu Lys Glu Tyr Pro Val Val Arg Tyr Leu Arg 505 510 515 520	1587
CAA CCA ATT TAT CTG GAA GTG AGA GTC CTA AAT AGG TCT GAC CCC AAC Gln Pro Ile Tyr Leu Glu Val Arg Val Leu Asn Arg Ser Asp Pro Asn 525 530 535	1635
ATC AAG CTG CTC TTA GAT GAC TGC TGG GCA ACA CCC ACG ATG GAC CCA Ile Lys Leu Val Leu Asp Asp Cys Trp Ala Thr Pro Thr Met Asp Pro 540 545 550	1683
GCC TCC GTC CCC CAG TGG AAT ATT ATC ATG GAT GGC TGT GAA TAC AAC Ala Ser Val Pro Gln Trp Asn Ile Ile Met Asp Gly Cys Glu Tyr Asn 555 560 565	1731
CTG GAC AAC CAC AGA ACC ACC TTC CAT CCA GTT GGC TCC TCT GTG ACC Leu Asp Asn His Arg Thr Thr Phe His Pro Val Gly Ser Ser Val Thr 570 575 580	1779
TAT CCT ACT CAC TAT CGG AGG TTT GAT GTG AAG ACC TTT GCC TTT GTC Tyr Pro Thr His Tyr Arg Arg Phe Asp Val Lys Thr Phe Ala Phe Val 585 590 595 600	1827
TCA GAG GCC CAA GTG CTT TCT AGT CTG GTC TAC TTC CAC TGC AGT GTC Ser Glu Ala Gln Val Leu Ser Ser Leu Val Tyr Phe His Cys Ser Val 605 610 615	1875
TTA ATC TGC AGT CGA CTG TCT GCT GAC TCC CCT CTG TGT TCC GTG ACT Leu Ile Cys Ser Arg Leu Ser Ala Asp Ser Pro Leu Cys Ser Val Thr 620 625 630	1923
TGC CCT GTG TCA TTC AGA CAC AGG AGA GCC ACA GGC ACC ACT GAA GAA Cys Pro Val Ser Phe Arg His Arg Arg Ala Thr Gly Thr Thr Glu Glu 635 640 645	1971
GAG AAA ATG ATA GTG AGT CTT CCA GGA CCC ATC CTC CTG CTG TCA GAT Glu Lys Met Ile Val Ser Leu Pro Gly Pro Ile Leu Leu Leu Ser Asp 650 655 660	2019
AGC TCT TCA CTC AGA GAT GTG GTG GAC TCA AAA GGG TAT GGG GCT GCC Ser Ser Ser Leu Arg Asp Val Val Asp Ser Lys Gly Tyr Gly Ala Ala 665 670 675 680	2067
GGA TAT GTT GCT TTT AAG ACT GTG GTA GCT GTG GCT GCC TTA GCA GGC Gly Tyr Val Ala Phe Lys Thr Val Val Ala Val Ala Ala Leu Ala Gly 685 690 695	2115
CTC GTG GCA ACG CTA GCC TTC ATC ACC TAC CTG CGC AAG AAC AGA ACC Leu Val Ala Thr Leu Gly Phe Ile Thr Tyr Leu Arg Lys Asn Arg Thr 700 705 710	2163
ATG ATA AAT CAC TAAGGATTTT CAATAAAAAT GGTTGAAGTA AAAAAAAA Met Ile Asn His 715	2215
AAAAAAAAAGCG GCGCGCAATT C	2236

(2) INFORMATION FOR SEQ ID NO:14:

- 97 -

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 716 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ala Ser Arg Gln Lys Gly Asp Ser Gly Ser Pro Ser Ser Trp Phe  
1 5 10 15  
Asn Ala Asp Trp Ser Thr Tyr Arg Ser Leu Phe Leu Leu Phe Ile Leu  
20 25 30  
Val Thr Ser Val Asn Ser Ile Gly Val Leu Gln Leu Val Asn Pro Val  
35 40 45  
Phe Pro Gly Thr Val Thr Cys Tyr Glu Thr Arg Met Ala Val Glu Phe  
50 55 60  
Pro Ser Asp Phe Gly Thr Lys Lys Trp His Thr Ser Val Val Asp Pro  
65 70 75 80  
Phe Ser Phe Glu Leu Leu Asn Cys Thr Tyr Ile Leu Asp Pro Glu Asn  
85 90 95  
Leu Thr Leu Lys Ala Pro Tyr Glu Thr Cys Thr Arg Arg Thr Leu Gly  
100 105 110  
Gln His Arg Met Ile Ile Arg Leu Lys Asp His Asn Ala Ala Ser Arg  
115 120 125  
His Asn Ser Leu Met Tyr Gln Ile Asn Cys Pro Val Met Gln Ala Glu  
130 135 140  
Glu Thr His Glu His Ala Gly Ser Thr Ile Cys Thr Lys Asp Ser Met  
145 150 155 160  
Ser Phe Thr Phe Asn Val Ile Pro Gly Leu Ala Asp Glu Asn Thr Asp  
165 170 175  
Ile Lys Asn Pro Met Gly Trp Ser Ile Glu Val Gly Asp Gly Thr Lys  
180 185 190  
Ala Lys Thr Leu Thr Leu Gln Asp Val Leu Arg Gln Gly Tyr Asn Ile  
195 200 205  
Leu Phe Asp Asn His Lys Ile Thr Phe Gln Val Ser Phe Asn Ala Thr  
210 215 220  
Gly Val Thr His Tyr Met Gln Gly Asn Ser His Leu Tyr Met Val Pro  
225 230 235 240  
Leu Lys Leu Ile His Glu Ser Leu Gly Gln Lys Ile Ile Leu Thr Thr  
245 250 255  
Arg Val Leu Cys Met Ser Asp Ala Val Thr Cys Asn Ala Thr His Val  
260 265 270  
Thr Leu Thr Ile Pro Glu Phe Pro Gly Lys Leu Lys Ser Val Ser Ser  
275 280 285  
Glu Asn Arg Asn Phe Ala Val Ser Gln Leu His Asn Asn Gly Ile Asp  
290 295 300  
Lys Glu Glu Ser Ser Gly Leu Thr Leu His Phe Ser Lys Thr Leu Leu

- 98 -

305	310	315	320
Lys Met Glu Phe Ser Glu Lys Cys Leu Pro Tyr Gln Phe Tyr Leu Ala			
325	330	335	
Ser Leu Lys Leu Thr Phe Ala Phe Asn Gln Glu Thr Ile Ser Thr Val			
340	345	350	
Leu Tyr Pro Glu Cys Val Cys Glu Ser Pro Val Ser Ile Val Thr Gly			
355	360	365	
Asp Leu Cys Thr Gln Asp Gly Phe Met Asp Ile Lys Val Tyr Ser His			
370	375	380	
Gln Thr Lys Pro Ala Leu Asn Leu Glu Thr Leu Arg Val Gly Asp Ser			
385	390	395	400
Ser Cys Gln Pro Thr Phe Gln Ala Ala Ser Gln Gly Leu Ile Leu Phe			
405	410	415	
His Ile Pro Leu Asn Gly Cys Gly Thr Arg His Lys Phe Lys Glu Gly			
420	425	430	
Lys Val Ile Tyr Glu Asn Glu Ile His Ala Val Trp Ala Asp Leu Pro			
435	440	445	
Pro Ser Thr Ile Ser Arg Asp Ser Glu Phe Arg Met Thr Val Gln Cys			
450	455	460	
His Tyr Ser Lys Gly Asp Leu Leu Ile Asn Thr Arg Val Gln Ser Leu			
465	470	475	480
Pro Pro Leu Glu Ala Ser Val Arg Pro Gly Pro Leu Ala Leu Ile Leu			
485	490	495	
Gln Thr Tyr Pro Asp Lys Ser Tyr Leu Gln Pro Tyr Gly Glu Lys Glu			
500	505	510	
Tyr Pro Val Val Arg Tyr Leu Arg Gln Pro Ile Tyr Leu Glu Val Arg			
515	520	525	
Val Leu Asn Arg Ser Asp Pro Asn Ile Lys Leu Val Leu Asp Asp Cys			
530	535	540	
Trp Ala Thr Pro Thr Met Asp Pro Ala Ser Val Pro Gln Trp Asn Ile			
545	550	555	560
Ile Met Asp Gly Cys Glu Tyr Asn Leu Asp Asn His Arg Thr Thr Phe			
565	570	575	
His Pro Val Gly Ser Ser Val Thr Tyr Pro Thr His Tyr Arg Arg Phe			
580	585	590	
Asp Val Lys Thr Phe Ala Phe Val Ser Glu Ala Gln Val Leu Ser Ser			
595	600	605	
Leu Val Tyr Phe His Cys Ser Val Leu Ile Cys Ser Arg Leu Ser Ala			
610	615	620	
Asp Ser Pro Leu Cys Ser Val Thr Cys Pro Val Ser Phe Arg His Arg			
625	630	635	640
Arg Ala Thr Gly Thr Thr Glu Glu Glu Lys Met Ile Val Ser Leu Pro			
645	650	655	
Gly Pro Ile Leu Leu Leu Ser Asp Ser Ser Ser Leu Arg Asp Val Val			
660	665	670	

- 99 -

Asp Ser Lys Gly Tyr Gly Ala Ala Gly Tyr Val Ala Phe Lys Thr Val  
675 680 685

Val Ala Val Ala Ala Leu Ala Gly Leu Val Ala Thr Leu Gly Phe Ile  
690 695 700

Thr Tyr Leu Arg Lys Asn Arg Thr Met Ile Asn His  
705 710 715

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1840 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Felis domesticus
  - (D) DEVELOPMENTAL STAGE: Juvenile
  - (E) HAPLOTYPE: Diploidy
  - (F) TISSUE TYPE: Ovary
  - (G) CELL TYPE: Oocyte

- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 57..1766

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAATTCCCGCG GCGCGCAAGTA CAGGTCTTGC AGCCACTGGG GGCTCCCGAT GGCATC	56
ATG TGG CTG CTG CAG CCC CTC TTG CTC TGT GTT CCC TTG TCT CTC GCT	104
Met Trp Leu Leu Gln Pro Leu Leu Leu Cys Val Pro Leu Ser Leu Ala	
1 5 10 15	
GTG CAT GGC CAG CAG AAG CCC CAG GTA CCA GAT TAT CCC GGT GAA CTC	152
Val His Gly Gln Gln Lys Pro Gln Val Pro Asp Tyr Pro Gly Glu Leu	
20 25 30	
CAT TGT GGG CTC CAG AGC CTT CAG TTT GCC ATA AAC CCG AGC CCC GGG	200
His Cys Gly Leu Gln Ser Leu Gln Phe Ala Ile Asn Pro Ser Pro Gly	
35 40 45	
AAA GCG ACT CCT GCA CTC ATA GTC TGG GAC AAT CGC GGG CTG CCA CAC	248
Lys Ala Thr Pro Ala Leu Ile Val Trp Asp Asn Arg Gly Leu Pro His	
50 55 60	
AAG CTG CAG AAC AAC TCT TGC TGC GGT ACC TGG GTA AGG GAG AGC CCG	296
Lys Leu Gln Asn Asn Ser Gly Cys Gly Thr Trp Val Arg Glu Ser Pro	
65 70 75 80	
GGG GGC TCC CTG CTG TTA GAC GCC TCT TAC AGC AGC TGC TAT GTC AAC	344
Gly Gly Ser Val Leu Leu Asp Ala Ser Tyr Ser Ser Cys Tyr Val Asn	
85 90 95	
GAG TGG GTG AGC ACG ACC CAA TCC CCA GGA AGC TCG AGG CCC CCC ACC	392
Glu Trp Val Ser Thr Thr Gln Ser Pro Gly Thr Ser Arg Pro Pro Thr	
100 105 110	

- 100 -

CCA GCA TCC AGG GTG ACT CCC CAG GAC TCC CAC TAC GTC ATG ATA GTC Pro Ala Ser Arg Val Thr Pro Gln Asp Ser His Tyr Val Met Ile Val 115	120	125	440
GGA CCTT GAA GGC ACA GAT GCG CCT GGG CGC AGG GTT ACC AAC ACC AAG Gly Val Glu Gly Thr Asp Ala Ala Gly Arg Arg Val Thr Asn Thr Lys 130	135	140	488
GTG CTC AGG TGT CCT AGG AAT CCC CCA GAC CAA GCT TTG GTG TCG AGC Val Leu Arg Cys Pro Arg Asn Pro Pro Asp Gln Ala Leu Val Ser Ser 145	150	155	536
TTA AGT CCC TCT CCT CTT CAA AAC GTA GCA CTA GAA GCT CCA AAC GCT Leu Ser Pro Ser Pro Leu Gln Asn Val Ala Leu Glu Ala Pro Asn Ala 165	170	175	584
GAC TTG TGT GAC TCT GTC CCA AAG TGG GAC AGG CTT CCG TGT GCT TCT Asp Leu Cys Asp Ser Val Pro Lys Trp Asp Arg Leu Pro Cys Ala Ser 180	185	190	632
TCA CCC ATC ACT CAG CGG GAC TGC AAT AAG CTT GGT TGC TGC TAC AAA Ser Pro Ile Thr Gln Gly Asp Cys Asn Lys Leu Gly Cys Cys Tyr Lys 195	200	205	680
TCA GAG GCA AAT TCC TGT TAC TAT GGA AAC ACA GTG ACC TCA CGC TGT Ser Glu Ala Asn Ser Cys Tyr Tyr Gly Asn Thr Val Thr Ser Arg Cys 210	215	220	728
ACC CAA GAC GGC CAC TTC ATC GCC GTG TCT CCG AAC GTG ACC TCA CGC TGT Thr Gln Asp Gly His Phe Ser Ile Ala Val Ser Arg Asn Val Thr Ser 225	230	235	776
CCC CCA CTG CTC TTA AAT TCT CTG CGC TTG GCC TTC GGG AAG GAC CGC Pro Pro Leu Leu Leu Asn Ser Leu Arg Leu Ala Phe Gly Lys Asp Arg 245	250	255	824
GAA TGT AAC CCT GTG AAA GCA ACA CGT GCC TTT GCC CTG TTC TTT TTT Glu Cys Asn Pro Val Lys Ala Thr Arg Ala Phe Ala Leu Phe Phe Phe 260	265	270	872
CCA TTT AAT TCC TGT GGC ACC ACG AGA TGG GTC ACT GCA GAC CAG GCA Pro Phe Asn Ser Cys Gly Thr Thr Arg Trp Val Thr Gly Asp Gln Ala 275	280	285	920
GTA TAT GAA AAT GAG CTG GTG GCA GCT AGA GAT GTG AGA ACT TGG AGC Val Tyr Glu Asn Glu Leu Val Ala Ala Arg Asp Val Arg Thr Trp Ser 290	295	300	968
CAT GGT TCT ATT ACC CGT GAC AGT ATC TTC AGG CTT CGA GTT AGC TGC His Gly Ser Ile Thr Arg Asp Ser Ile Phe Arg Leu Arg Val Ser Cys 305	310	315	1016
AGC TAC TCT GTA AGG AGT AAT GCC TTC CCG CTT AGC GTT CAG GTG TTT Ser Tyr Ser Val Arg Ser Asn Ala Phe Pro Leu Ser Val Gln Val Phe 325	330	335	1064
ACC ATC CCA CCA CCC CAT CTG AAA ACC CAG CAT GGA CCC CTC ACT CTG Thr Ile Pro Pro His Leu Lys Thr Gln His Gly Pro Leu Thr Leu 340	345	350	1112
GAA CTC AAG ATT GCC AAA GAT AAG CAC TAT GGC TCC TAC TAC ACT ATT Glu Leu Lys Ile Ala Lys Asp Lys His Tyr Gly Ser Tyr Tyr Thr Ile 355	360	365	1160
GGT GAC TAC CCA CTG GTA AAG TTG CTT CCG GAT CCC ATT TAT GTG GAG Gly Asp Tyr Pro Val Val Lys Leu Leu Arg Asp Pro Ile Tyr Val Glu 370	375	380	1208

- 101 -

GTC TCT ATC CGC CAC AGA ACG GAC CCC TCC CTG GGG CTG CTC CTC CAT Val Ser Ile Arg His Arg Thr Asp Pro Ser Leu Gly Leu Leu Leu His 385 390 395 400	1256
AAC TGT TGG GCC ACA CCC GGC AAC TAC TCC CAG AGT CTG TCC CAG TGG Asn Cys Trp Ala Thr Pro Gly Lys Asn Ser Gln Ser Leu Ser Gln Trp 405 410 415	1304
CCC ATT CTG GTG AAA GGA TGC CCC TAC GTT GGA GAC AAC TAT CAA ACC Pro Ile Leu Val Lys Gly Cys Pro Tyr Val Gly Asp Asn Tyr Gln Thr 420 425 430	1352
CAG CTG ATC CCT GTC CAG AAG GCT CTG GAT ACA CCA TTT CCA TCT TAC Gln Leu Ile Pro Val Gln Lys Ala Leu Asp Thr Pro Phe Pro Ser Tyr 435 440 445	1400
TAC AAG CCC TTC AGT ATT TTC ACC TTC AGC TTT GTG GAC ACC ATG GCA Tyr Lys Arg Phe Ser Ile Phe Thr Phe Ser Phe Val Asp Thr Met Ala 450 455 460	1448
AAG TGG GCA CTC AGG GGA CCG GTG TAT CTG CAC TGT AAT GTA TCC ATC Lys Trp Ala Leu Arg Gly Pro Val Tyr Leu His Cys Asn Val Ser Ile 465 470 475 480	1496
TGC CAG CCT GCT GGG ACC TCC TCC TGT AGG ATA ACC TGT CCT GTT GCC Cys Gln Pro Ala Gly Thr Ser Ser Cys Arg Ile Thr Cys Pro Val Ala 485 490 495	1544
AGG CGA AGA AGA CAC TCT GAC CTC CAT CAT CRC AGC AGT ACT GCG AGC Arg Arg Arg Arg His Ser Asp Leu His His Ser Ser Thr Ala Ser 500 505 510	1592
ATC TCT AGC AAG GGT CCC ATG ATT CTA CTC CAA GCC ACT ATG GAC TCT Ile Ser Ser Lys Gly Pro Met Ile Leu Leu Gln Ala Thr Met Asp Ser 515 520 525	1640
GCA GAG AAG CTC CAC AAA AAC TCA AGT TCT CCT ATA GAC TCC CAA GCT Ala Glu Lys Leu His Lys Asn Ser Ser Ser Pro Ile Asp Ser Gln Ala 530 535 540	1688
CTG TGG ATG GCA GGC CTT TCC GGG ACC CTA ATC TTT GGA TTC TTG TTA Leu Trp Met Ala Gly Leu Ser Gly Thr Leu Ile Phe Gly Phe Leu Leu 545 550 555 560	1736
GTC TCC TAC TTG GCT ATC AGG AAA CGG AGG TGAATTATTC CAGTTGTGTT Val Ser Tyr Leu Ala Ile Arg Lys Arg Arg 565 570	1786
AATAAAACCA GATTGCATTA CCAAAAAAA AAAAAAAA CGGGCCGCGA ATTC	1840

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 570 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Trp Leu Leu Gln Pro Leu Leu Leu Cys Val Pro Leu Ser Leu Ala	
1                         5                         10                         15	
Val His Gly Gln Gln Lys Pro Gln Val Pro Asp Tyr Pro Gly Glu Leu	
20                         25                         30	

- 102 -

His Cys Gly Leu Gln Ser Leu Gln Phe Ala Ile Asn Pro Ser Pro Gly  
35 40 45

Lys Ala Thr Pro Ala Leu Ile Val Trp Asp Asn Arg Gly Leu Pro His  
50 55 60

Lys Leu Gln Asn Asn Ser Gly Cys Gly Thr Trp Val Arg Glu Ser Pro  
65 70 75 80

Gly Gly Ser Val Leu Leu Asp Ala Ser Tyr Ser Ser Cys Tyr Val Asn  
85 90 95

Glu Trp Val Ser Thr Thr Gln Ser Pro Gly Thr Ser Arg Pro Pro Thr  
100 105 110

Pro Ala Ser Arg Val Thr Pro Gln Asp Ser His Tyr Val Met Ile Val  
115 120 125

Gly Val Glu Gly Thr Asp Ala Ala Gly Arg Arg Val Thr Asn Thr Lys  
130 135 140

Val Leu Arg Cys Pro Arg Asn Pro Pro Asp Gln Ala Leu Val Ser Ser  
145 150 155 160

Leu Ser Pro Ser Pro Leu Gln Asn Val Ala Leu Glu Ala Pro Asn Ala  
165 170 175

Asp Leu Cys Asp Ser Val Pro Lys Trp Asp Arg Leu Pro Cys Ala Ser  
180 185 190

Ser Pro Ile Thr Gln Gly Asp Cys Asn Lys Leu Gly Cys Cys Tyr Lys  
195 200 205

Ser Glu Ala Asn Ser Cys Tyr Tyr Gly Asn Thr Val Thr Ser Arg Cys  
210 215 220

Thr Gln Asp Gly His Phe Ser Ile Ala Val Ser Arg Asn Val Thr Ser  
225 230 235 240

Pro Pro Leu Leu Leu Asn Ser Leu Arg Leu Ala Phe Gly Lys Asp Arg  
245 250 255

Glu Cys Asn Pro Val Lys Ala Thr Arg Ala Phe Ala Leu Phe Phe Phe  
260 265 270

Pro Phe Asn Ser Cys Gly Thr Thr Arg Trp Val Thr Gly Asp Gln Ala  
275 280 285

Val Tyr Glu Asn Glu Leu Val Ala Ala Arg Asp Val Arg Thr Trp Ser  
290 295 300

His Gly Ser Ile Thr Arg Asp Ser Ile Phe Arg Leu Arg Val Ser Cys  
305 310 315 320

Ser Tyr Ser Val Arg Ser Asn Ala Phe Pro Leu Ser Val Gln Val Phe  
325 330 335

Thr Ile Pro Pro Pro His Leu Lys Thr Gln His Gly Pro Leu Thr Leu  
340 345 350

Glu Leu Lys Ile Ala Lys Asp Lys His Tyr Gly Ser Tyr Tyr Thr Ile  
355 360 365

Gly Asp Tyr Pro Val Val Lys Leu Leu Arg Asp Pro Ile Tyr Val Glu  
370 375 380

Val Ser Ile Arg His Arg Thr Asp Pro Ser Leu Gly Leu Leu Leu His

- 103 -

385	390	395	400
Asn Cys Trp Ala Thr Pro Gly Lys Asn Ser Gln Ser Leu Ser Gln Trp			
405		410	415
Pro Ile Leu Val Lys Gly Cys Pro Tyr Val Gly Asp Asn Tyr Gln Thr			
420	425		430
Gln Leu Ile Pro Val Gln Lys Ala Leu Asp Thr Pro Phe Pro Ser Tyr			
435	440	445	
Tyr Lys Arg Phe Ser Ile Phe Thr Phe Ser Val Asp Thr Met Ala			
450	455	460	
Lys Trp Ala Leu Arg Gly Pro Val Tyr Leu His Cys Asn Val Ser Ile			
465	470	475	480
Cys Gln Pro Ala Gly Thr Ser Ser Cys Arg Ile Thr Cys Pro Val Ala			
485	490	495	
Arg Arg Arg Arg His Ser Asp Leu His His Ser Ser Thr Ala Ser			
500	505	510	
Ile Ser Ser Lys Gly Pro Met Ile Leu Leu Gln Ala Thr Met Asp Ser			
515	520	525	
Ala Glu Lys Leu His Lys Asn Ser Ser Ser Pro Ile Asp Ser Gln Ala			
530	535	540	
Leu Trp Met Ala Gly Leu Ser Gly Thr Leu Ile Phe Gly Phe Leu Leu			
545	550	555	560
Val Ser Tyr Leu Ala Ile Arg Lys Arg Arg			
565	570		

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1319 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: *Felis domesticus*
  - (D) DEVELOPMENTAL STAGE: Juvenile
  - (E) HAPLOTYPE: Diploidy
  - (F) TISSUE TYPE: Ovary
  - (G) CELL TYPE: Oocyte
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 26..1297

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
GAATTGGCGG CGGCCCGTAG GCCGC ATG GGG CTG AGC TAC GGG CTT TTC ATC
Met Gly Leu Ser Tyr Gly Leu Phe Ile
1 5
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- 104 -

TGT TTT CTG CTT TGG GCA GGC ACG GGG CTG TGC TAT CCC CCA ACC ACC Cys Phe Leu Leu Trp Ala Gly Thr Gly Leu Cys Tyr Pro Pro Thr Thr 10 15 20 25	100
ACC GAG GAT AAC ACC CAC CCC TCG TTG CCA TCA AGC CCC TCT GTG GTG Thr Glu Asp Lys Thr His Pro Ser Leu Pro Ser Ser Pro Ser Val Val 30 35 40	148
GTA GAG TGT CGG CAT GCC TGG CTG GTG GTC AAC GTC AGC AAA AAC CTT Val Glu Cys Arg His Ala Trp Leu Val Val Asn Val Ser Lys Asn Leu 45 50 55	196
TTT GGT ACT GGG AGG CTT GTG AGG CCT GCA GAC CTC ACC CTG GGT CCG Phe Gly Thr Gly Arg Leu Val Arg Pro Ala Asp Leu Thr Leu Gly Pro 60 65 70	244
GAG AAC TGT GAG CCC CTG ATC TCT GGG GAC TCA GAT GAT ACG GTC AGG Glu Asn Cys Glu Pro Leu Ile Ser Gly Asp Ser Asp Asp Thr Val Arg 75 80 85	292
TTT GAA GTC GAG CTC CAC AAG TGT GGC AAC AGC GTG CAG GTG ACC GAA Phe Glu Val Glu Leu His Lys Cys Gly Asn Ser Val Gln Val Thr Glu 90 95 100 105	340
GAT GCC CTG GTG TAT AGC ACC TTC CTG CTC CAC AAC CCC CGC CCC ATG Asp Ala Leu Val Tyr Ser Thr Phe Leu Leu His Asn Pro Arg Pro Met 110 115 120	388
GGA AAC CTG TCC ATC CTG AGG ACC AAC CGC GCG GAA GTT CCC ATT GAG Gly Asn Leu Ser Ile Leu Arg Thr Asn Arg Ala Glu Val Pro Ile Glu 125 130 135	436
TGC CGT TAC CCC AGG CAT AGC AAC GTG AGC AGC GAG GCC ATC CTG CCC Cys Arg Tyr Pro Arg His Ser Asn Val Ser Ser Glu Ala Ile Leu Pro 140 145 150	484
ACC TGG GTG CCC TTC AGG ACC ACA ATG CTC TCA GAG GAG RAG ATG CTG GCT Thr Trp Val Pro Phe Arg Thr Met Leu Ser Glu Glu Lys Leu Ala 155 160 165	532
TTC TCT CTG CGC CTG ATG GAG GAG GAC TGG GGC TCC GAG AAG CAG TCC Phe Ser Leu Arg Leu Met Glu Glu Asp Trp Gly Ser Glu Lys Gln Ser 170 175 180 185	580
CCC ACT TTC CAG TTG GGA GAC CTA GCC CAC CTC CAG GCC GAA GTC CAC Pro Thr Phe Gln Leu Gly Asp Leu Ala His Leu Gln Ala Glu Val His 190 195 200	628
ACC GGC CGC CAC ATA CCA CTG CGA CTG TTT GTG GAC TAC TGT GTG GCC Thr Gly Arg His Ile Pro Leu Arg Leu Phe Val Asp Tyr Cys Val Ala 205 210 215	676
ACG CTG ACA CCA GAC CAG AAC GCC TCC CCT CAT CAC ACC ATC GTG GAC Thr Leu Thr Pro Asp Gln Asn Ala Ser Pro His His Thr Ile Val Asp 220 225 230	724
TTC CAC GGC TGT CTC GTG GAT GGT CTC TCT GAT GCC TCT TCT GCC TTC Phe His Gly Cys Leu Val Asp Gly Leu Ser Asp Ala Ser Ser Ala Phe 235 240 245	772
AAA GCC CCC AGA CCC AGG CCG GAG ACT CTC CAG TTT ACA GTC GAC ACC Lys Ala Pro Arg Pro Arg Pro Glu Thr Leu Gln Phe Thr Val Asp Thr 250 255 260 265	820
TTC CAC TTT GCT AAT GAC CCC AGA AAC ATG ATC TAT ACC TGC CAT Phe His Phe Ala Asn Asp Pro Arg Asn Met Ile Tyr Ile Thr Cys His 270 275 280	868

- 105 -

CTG AAA GTC ACT CCA GCT ACC CGA GTC CCA GAC CAG CTA AAC AAA GCC Leu Lys Val Thr Pro Ala Ser Arg Val Pro Asp Gln Leu Asn Lys Ala 285 290 295	916
TGT TCC TTC ATC AAG TCT TCT AAC AGG TGG TTC CCA GTA GAA GGC CCT Cys Ser Phe Ile Lys Ser Ser Asn Arg Trp Phe Pro Val Glu Gly Pro 300 305 310	964
GCT GAC ATC TGT AAC TGT TGT AAC AAA GGT AGC TGT GGC CTT CAG GGC Ala Asp Ile Cys Asn Cys Cys Asn Lys Glu Ser Cys Gly Leu Gln Gly 315 320 325	1012
CGT TCC TGG AGG CTG TCC CAC CTA GAC AGA CCG TGG CRC AAG ATG GCT Arg Ser Trp Arg Leu Ser His Leu Asp Arg Pro Trp His Lys Met Ala 330 335 340 345	1060
TCC CGA AAT CGC AGG CAT GTG ACC GAA GAA GCG GAT ATC ACC CTG GGG Ser Arg Asn Arg His Val Thr Glu Ala Asp Ile Thr Val Glu 350 355 360	1108
CCT CTG ATC TTC CTG GGA AAG GCT GCC GAT CGT GGT GTG GAG GGG TCG Pro Leu Ile Phe Leu Gly Lys Ala Ala Asp Arg Gly Val Glu Gly Ser 365 370 375	1156
ACC TCG CCT CAC ACC TCT CTG ATC GTG GGC ATA GCC CTG CCC ACG GTC Thr Ser Pro His Thr Ser Val Met Val Gly Ile Gly Leu Ala Thr Val 380 385 390	1204
TTG TCC CTG ACT CTG GCT ACC ATT GTC CTG GGT CTC GCC AGG AGG CAT Leu Ser Leu Thr Leu Ala Thr Ile Val Leu Gly Leu Ala Arg Arg His 395 400 405	1252
CAC ACT GCT TCC CGT CCT ATT ATC TGC CCT CTG TCT GCT TCC CAA His Thr Ala Ser Arg Pro Met Ile Cys Pro Val Ser Ala Ser Gln 410 415 420	1297
<b>AAAAAGAACGGCCCGGAAT TC</b>	
	1319

## (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 424 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Gly Leu Ser Tyr Gly Leu Phe Ile Cys Phe Leu Leu Trp Ala Gly 1 5 10 15
Thr Gly Leu Cys Tyr Pro Pro Thr Thr Thr Glu Asp Lys Thr His Pro 20 25 30
Ser Leu Pro Ser Ser Pro Ser Val Val Val Glu Cys Arg His Ala Trp 35 40 45
Leu Val Val Asn Val Ser Lys Asn Leu Phe Gly Thr Gly Arg Leu Val 50 55 60
Arg Pro Ala Asp Leu Thr Leu Gly Pro Glu Asn Cys Glu Pro Leu Ile 65 70 75 80
Ser Gly Asp Ser Asp Asp Thr Val Arg Phe Glu Val Glu Leu His Lys 85 90 95

- 106 -

Cys Gly Asn Ser Val Gln Val Thr Glu Asp Ala Leu Val Tyr Ser Thr  
100 105 110  
Phe Leu Leu His Asn Pro Arg Pro Met Gly Asn Leu Ser Ile Leu Arg  
115 120 125  
Thr Asn Arg Ala Glu Val Pro Ile Glu Cys Arg Tyr Pro Arg His Ser  
130 135 140  
Asn Val Ser Ser Glu Ala Ile Leu Pro Thr Trp Val Pro Phe Arg Thr  
145 150 155 160  
Thr Met Leu Ser Glu Glu Lys Leu Ala Phe Ser Leu Arg Leu Met Glu  
165 170 175  
Glu Asp Trp Gly Ser Glu Lys Gln Ser Pro Thr Phe Gln Leu Gly Asp  
180 185 190  
Leu Ala His Leu Gln Ala Glu Val His Thr Gly Arg His Ile Pro Leu  
195 200 205  
Arg Leu Phe Val Asp Tyr Cys Val Ala Thr Leu Thr Pro Asp Gln Asn  
210 215 220  
Ala Ser Pro His His Thr Ile Val Asp Phe His Gly Cys Leu Val Asp  
225 230 235 240  
Gly Leu Ser Asp Ala Ser Ser Ala Phe Lys Ala Pro Arg Pro Arg Pro  
245 250 255  
Glu Thr Leu Gln Phe Thr Val Asp Thr Phe His Phe Ala Asn Asp Pro  
260 265 270  
Arg Asn Met Ile Tyr Ile Thr Cys His Leu Lys Val Thr Pro Ala Ser  
275 280 285  
Arg Val Pro Asp Gln Leu Asn Lys Ala Cys Ser Phe Ile Lys Ser Ser  
290 295 300  
Asn Arg Trp Phe Pro Val Glu Gly Pro Ala Asp Ile Cys Asn Cys Cys  
305 310 315 320  
Asn Lys Gly Ser Cys Gly Leu Gln Gly Arg Ser Trp Arg Leu Ser His  
325 330 335  
Leu Asp Arg Pro Trp His Lys Met Ala Ser Arg Asn Arg Arg His Val  
340 345 350  
Thr Glu Glu Ala Asp Ile Thr Val Gly Pro Leu Ile Phe Leu Gly Lys  
355 360 365  
Ala Ala Asp Arg Gly Val Glu Gly Ser Thr Ser Pro His Thr Ser Val  
370 375 380  
Met Val Gly Ile Gly Leu Ala Thr Val Leu Ser Leu Thr Leu Ala Thr  
385 390 395 400  
Ile Val Leu Gly Leu Ala Arg Arg His His Thr Ala Ser Arg Pro Met  
405 410 415  
Ile Cys Pro Val Ser Ala Ser Gln  
420

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 643 base pairs

- 107 -

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Bos taurus  
 (D) DEVELOPMENTAL STAGE: Juvenile  
 (E) HAPLOTYPE: Diploid  
 (F) TISSUE TYPE: Ovary  
 (G) CELL TYPE: Oocyte

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 16..582

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCGCCG CGCCCT AAT AAC AGG ACT GAC CCC AAC ATC AAG TTG GTC TTA	51
Leu Asn Arg Thr Asp Pro Asn Ile Lys Leu Val Leu	
1 5 10	
GAT TGC TGG GCA ACA TCC ACC ATG GAC CCA GCC TCT CTC CCT CAG	99
Asp Cys Trp Ala Thr Ser Thr Met Asp Pro Ala Ser Leu Pro Gin	
15 20 25	
AAT ATT ATC GTG GAT GGC TGT GAA TAC AAC TTG GAC AAC CAC AGA	147
Asn Ile Ile Val Asp Gly Cys Glu Tyr Asn Leu Asp Asn His Arg	
30 35 40	
ACC TTC CAT CCG GTT GGC TCC TCG GTG GCC TAT CCT AAT CAC TAC	195
Thr Phe His Pro Val Gly Ser Ser Val Ala Tyr Pro Asn His Tyr	
50 55 60	
AGG TTT GCT GTG AAG ACC TTT GCC TTT GTG TCA GAG GAC CCG CGC	243
Arg Phe Ala Val Lys Thr Phe Ala Phe Val Ser Glu Asp Pro Ala	
65 70 75	
TCT CAC TTG GTC TAC TTC CAC TGC AGC GCC TTA ATC TGC GAT CAA	291
Ser His Leu Val Tyr Phe His Cys Ser Ala Leu Ile Cys Asp Gln	
80 85 90	
TCT TCT AAC TTC CCC CTG TGT TCT GCG TCT TGC CTT GTG TCA TCC	339
Ser Ser Asn Phe Pro Leu Cys Ser Ala Ser Cys Leu Val Ser Ser	
95 100 105	
AGC AGG CGA GCC ACA GGG GCC ACT GAG GAA GAG AAG ATG ATA GTG	387
Ser Arg Arg Ala Thr Gly Ala Thr Glu Glu Lys Met Ile Val	
110 115 120	
TCT CCG GGC CCC ATC CTC CTG TTG TCA GAT GGC TCT TCA TTC AGA	435
Leu Pro Gly Pro Ile Leu Leu Ser Asp Gly Ser Ser Phe Arg	
130 135 140	
TCT GTG GAT TCT AAA GGG CAT GGG ACT TCT GGA TAT GCT GCT TTT	483
Ile Val Asp Ser Lys Gly His Gly Thr Ser Gly Tyr Ala Ala Phe	
145 150 155	
ACT ATG GTT GCT GTA CTT GCC TTA GCA GGT GTT GTG GCA ACT CTA	531
Thr Met Val Ala Val Val Ala Leu Ala Gly Val Val Ala Thr Leu	
160 165 170	

- 108 -

AGC CTA ATC AGC TAC CTG CGC AAG AAA AGA ATC ACA GTG CTA AAC CAC Ser Leu Ile Ser Tyr Leu Arg Lys Lys Arg Ile Thr Val Leu Asn His 175 180 185	579
TAATTGGATT TTCAATAAAA TGTGGAAAGTA AAAAAAAA AAAAAAAA GCGGCCCGA	639
ATTC	643

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 188 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

Leu Asn Arg Thr Asp Pro Asn Ile Lys Leu Val Leu Asp Asp Cys Trp
1 5 10 15
Ala Thr Ser Thr Met Asp Pro Ala Ser Leu Pro Gln Trp Asn Ile Ile
20 25 30
Val Asp Gly Cys Glu Tyr Asn Leu Asp Asn His Arg Thr Thr Phe His
35 40 45
Pro Val Gly Ser Ser Val Ala Tyr Pro Asn His Tyr Gln Arg Phe Ala
50 55 60
Val Lys Thr Phe Ala Phe Val Ser Glu Asp Pro Ala Phe Ser His Leu
65 70 75 80
Val Tyr Phe His Cys Ser Ala Leu Ile Cys Asp Gln Leu Ser Ser Asn
85 90 95
Phe Pro Leu Cys Ser Ala Ser Cys Leu Val Ser Ser Arg Ser Arg Arg
100 105 110
Ala Thr Gly Ala Thr Glu Glu Lys Met Ile Val Ser Leu Pro Gly
115 120 125
Pro Ile Leu Leu Leu Ser Asp Gly Ser Ser Phe Arg Asp Ala Val Asp
130 135 140
Ser Lys Gly His Gly Thr Ser Gly Tyr Ala Ala Phe Lys Thr Met Val
145 150 155 160
Ala Val Val Ala Leu Ala Gly Val Val Ala Thr Leu Ser Leu Ile Ser
165 170 175
Tyr Leu Arg Lys Lys Arg Ile Thr Val Leu Asn His
180 185

```

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1029 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- 109 -

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bos taurus
- (D) DEVELOPMENTAL STAGE: Juvenile
- (E) HAPLOTYPE: Diploid
- (F) TISSUE TYPE: Ovary
- (G) CELL TYPE: Oocyte

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..976

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

G AAT TCT GTA CAC TTG GCC TTC AGG AAT GAC AGC GAA TGT AAA CCT Asn Ser Val His Leu Ala Phe Arg Asn Asp Ser Glu Cys Lys Pro 1 5 10 15	46
GTG ATG GCA ACA CAC ACT TTT GTT CTG TTC CCG TTT CCA TTT ACT ACT Val Met Ala Thr His Thr Phe Val Leu Phe Arg Phe Pro Phe Thr Thr 20 25 30	94
TGT GGT ACT ACA AAA CAG ATC ACT GGA AAG CAA GCG GTA TAT GAA AAT Cys Gly Thr Thr Lys Gln Ile Thr Gly Lys Gln Ala Val Tyr Glu Asn 35 40 45	142
GAG CTG GTT GCA GCT CGG GAT GTG AGA ACT TGG AGC CGT GGT TCT ATT Glu Leu Val Ala Ala Arg Asp Val Arg Thr Trp Ser Arg Gly Ser Ile 50 55 60	190
ACC CGA GAC AGT ACC TTC AGG CTC CAA GTC AGT TGT AGC TAC TCT GCA Thr Arg Asp Ser Thr Phe Arg Leu Gln Val Ser Cys Ser Tyr Ser Ala 65 70 75	238
AGT AGC ACT GCT CTC CCA GTT ATT GTC CAA GTT CTT ACT CTC CCA CCA Ser Ser Ser Ala Leu Pro Val Asn Val Gln Val Leu Thr Leu Pro Pro 80 85 90 95	286
CCC CCT CCT GAG ACC CTG CCT GGA AAC CTC ACT CTG GAA CTT AAG ATT Pro Leu Pro Glu Thr Leu Pro Gly Asn Leu Thr Leu Glu Leu Lys Ile 100 105 110	334
GCC AAA GAT AAA CCG TAT CGC TCC TAC TAC ACG GCT AGT GAC TAC CCA Ala Lys Asp Lys Pro Tyr Arg Ser Tyr Tyr Thr Ala Ser Asp Tyr Pro 115 120 125	382
GTG CTG AAC TTA CTT CGG GAT CCC ATC TAC GTG GAA GTC TCC ATC CAT Val Val Lys Leu Leu Arg Asp Pro Ile Tyr Val Glu Val Ser Ile His 130 135 140	430
CAG AGA ACA GAC CCC AGT CTC GAG CTG CGC CTG GAC CAG TGT TGG GCG Gln Arg Thr Asp Pro Ser Leu Glu Leu Arg Leu Asp Gln Cys Trp Ala 145 150 155	478
ACA CCT GGT GCA GAT GCC CTG CTC CAG CCC CAG TGG CCC TTG CTT GTG Thr Pro Gly Ala Asp Ala Leu Leu Gln Pro Gln Trp Pro Leu Leu Val 160 165 170 175	526
AAT GGG TGC CCC TAC ACA GGA GAC AAC TAT CAG ACA AAA CTG ATC CCT Asn Gly Cys Pro Tyr Thr Gly Asp Asn Tyr Gln Thr Lys Leu Ile Pro 180 185 190	574

- 110 -

GTC TGG GAA GCC TCA GAC CTG CCG TTT CCT TCT CAC TAC CAG CGC TTC Val Trp Glu Ala Ser Asp Leu Pro Phe Pro Ser His Tyr Gln Arg Phe 195 200 205	622
AGC ATT TCC ACC TTC AGC TTT GTG GAC TCA GTG GCA AAG CGG GCC CTC Ser Ile Ser Thr Phe Ser Phe Val Asp Ser Val Ala Lys Arg Ala Leu 210 215 220	670
AAG GGA CGG GTG TAT CTG CAC TCC AGT GCA TCG GTC TGC CAG CCT GCC Lys Gly Pro Val Tyr Leu His Cys Ser Ala Ser Val Cys Gln Pro Ala 225 230 235	718
GGG ACA CCA TCC TGT GTG ACA CTC TGT CCT GCC AGA CGA AGA AGA AGC Gly Thr Pro Ser Cys Val Thr Leu Cys Pro Ala Arg Arg Arg Arg Ser 240 245 250 255	766
TCT GAC ATC CAT TTT CAG AAC AAA ACG GCT AGC ATT TCT AGC AAG GGT Ser Asp Ile His Phe Gln Asn Lys Thr Ala Ser Ile Ser Ser Lys Gly 260 265 270	814
CCC TTG ATT CTA CTC CAA GCC ATT CAA GAC TCT TCA GAA AAG CTC CAC Pro Leu Ile Leu Leu Gln Ala Ile Gln Asp Ser Ser Glu Lys Leu His 275 280 285	862
AAA TAC TCA AGG TCT CCT GTC GAC TCT CAA GCT TTG TGG GTC GCT GCC Lys Tyr Ser Arg Ser Pro Val Asp Ser Gln Ala Leu Trp Val Ala Gly 290 295 300	910
CTA TCT GGA ATC TTA ATC GTT GGA GCC TTG TTC ATG TCC TAC CTG GCC Leu Ser Gly Ile Leu Ile Val Gly Ala Leu Phe Met Ser Tyr Leu Ala 305 310 315	958
ATT AGG AAA TGG AGA TGAGTTGCTC AGCCCCAATGG TGTAAATAAAA ACCAGATTGC Ile Arg Lys Trp Arg 320	1013
AGCCGGCCGC GAAATTC	1029
 (2) INFORMATION FOR SEQ ID NO:22:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 324 amino acids	
(B) TYPE: amino acid	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: protein	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:	
Asn Ser Val His Leu Ala Phe Arg Asn Asp Ser Glu Cys Lys Pro Val 1 5 10 15	
Met Ala Thr His Thr Phe Val Leu Phe Arg Phe Pro Phe Thr Thr Cys 20 25 30	
Gly Thr Thr Lys Gln Ile Thr Gly Lys Gln Ala Val Tyr Glu Asn Glu 35 40 45	
Leu Val Ala Ala Arg Asp Val Arg Thr Trp Ser Arg Gly Ser Ile Thr 50 55 60	
Arg Asp Ser Thr Phe Arg Leu Gln Val Ser Cys Ser Tyr Ser Ala Ser 65 70 75 80	
Ser Ser Ala Leu Pro Val Asn Val Gln Val Leu Thr Leu Pro Pro Pro 85 90 95	

- 111 -

Leu Pro Glu Thr Leu Pro Gly Asn Leu Thr Leu Glu Leu Lys Ile Ala  
100 105 110

Lys Asp Lys Pro Tyr Arg Ser Tyr Tyr Thr Ala Ser Asp Tyr Pro Val  
115 120 125

Val Lys Leu Leu Arg Asp Pro Ile Tyr Val Glu Val Ser Ile His Gln  
130 135 140

Arg Thr Asp Pro Ser Leu Glu Leu Arg Leu Asp Gln Cys Trp Ala Thr  
145 150 155 160

Pro Gly Ala Asp Ala Leu Leu Gln Pro Gln Trp Pro Leu Leu Val Asn  
165 170 175

Gly Cys Pro Tyr Thr Gly Asp Asn Tyr Gln Thr Lys Leu Ile Pro Val  
180 185 190

Trp Glu Ala Ser Asp Leu Pro Phe Pro Ser His Tyr Gln Arg Phe Ser  
195 200 205

Ile Ser Thr Phe Ser Phe Val Asp Ser Val Ala Lys Arg Ala Leu Lys  
210 215 220

Gly Pro Val Tyr Leu His Cys Ser Ala Ser Val Cys Gln Pro Ala Gly  
225 230 235 240

Thr Pro Ser Cys Val Thr Leu Cys Pro Ala Arg Arg Arg Ser Ser  
245 250 255

Asp Ile His Phe Gln Asn Lys Thr Ala Ser Ile Ser Ser Lys Gly Pro  
260 265 270

Leu Ile Leu Leu Gln Ala Ile Gln Asp Ser Ser Glu Lys Leu His Lys  
275 280 285

Tyr Ser Arg Ser Pro Val Asp Ser Gln Ala Leu Trp Val Ala Gly Leu  
290 295 300

Ser Gly Ile Leu Ile Val Gly Ala Leu Phe Met Ser Tyr Leu Ala Ile  
305 310 315 320

Arg Lys Trp Arg

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1457 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bos taurus
- (D) DEVELOPMENTAL STAGE: Juvenile
- (E) HAPLOTYPE: Diploid
- (F) TISSUE TYPE: Ovary
- (G) CELL TYPE: Oocyte

- 112 -

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 149..1411

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCGGGGCCTC CCTACTCTCA GGAAGGCACC CGCTCACCTC CTCAAGTTCT CGATCTGGC	60
CGGGATGCTC TGAAGCTGGT TGCCCCGAG GCTGAGGGTC TGCAAGGGCG CAGTCCAGCA	120
GGCAGGGTGGG AGTGGCTTCG TGGGCACC ATG GGG CCG TGC TCT AGG CTG TTC Met Gly Pro Cys Ser Arg Leu Phe	172
1                               5	
GTC TGC TTT CTG CTC TCG GGA AGC ACA GAG CTC TGC AGC CCC CAG CCC Val Cys Phe Leu Leu Trp Gly Ser Thr Glu Leu Cys Ser Pro Gln Pro	220
10                             15                               20	
TTC TGG GAT GAT GAA ACC GAG CGC TTC AGG CCA TCA AAG CCG CCC GCC Phe Trp Asp Asp Glu Thr Glu Arg Phe Arg Pro Ser Lys Pro Pro Ala	268
25                             30                             35                             40	
GTG ATC GTG GAG TGT CAG GAG GCC CAG CTG GTG GTC ACA GTC GAC AAA Val Met Val Glu Cys Glu Ala Gln Leu Val Val Thr Val Asp Lys	316
45                             50                             55	
GAC CTT TTC GGC ACA GGG AAG CTC ATC CGG CCT GCG GAC CTC ACC CTG Asp Leu Phe Gly Thr Gly Lys Leu Ile Arg Pro Ala Asp Leu Thr Leu	364
60                             65                             70	
GGC CCC GAC AAC TGT GAG CCG CTG GCC TCC CGC GAC ACC GAT GGC GTG Gly Pro Asp Asn Cys Glu Pro Leu Ala Ser Ala Asp Thr Asp Gly Val	412
75                             80                             85	
GTT AGG TTT GCG GTC GGG CTG CAC GAG TGT GGC AAC ATC TTG CAG GTG Val Arg Phe Ala Val Gly Leu His Glu Cys Gly Asn Ile Leu Gln Val	460
90                             95                             100	
ACC GAC AAT GCC CTG GTG TAC AGC ACC TTC CTG CTC CAC AAC CCC CGC Thr Asp Asn Ala Leu Val Tyr Ser Thr Phe Leu Leu His Asn Pro Arg	508
105                             110                             115                             120	
CCT GCA GGA AAC CTG TCC ATC CTG AGG ACT AAC CGC GCA GAG GTC CCC Pro Ala Gly Asn Leu Ser Ile Leu Arg Thr Asn Arg Ala Glu Val Pro	556
125                             130                             135	
ATC GAG TGC CAC TAC CCC AGG CAG GGC AAT GTG AGT AGC TGG GCC ATC Ile Glu Cys His Pro Arg Gln Gly Asn Val Ser Ser Trp Ala Ile	604
140                             145                             150	
CAG CCC ACC TGG GTG CCA TTC AGG ACC ACA GTG TTC TCG GAG GAG RAG Gln Pro Thr Trp Val Pro Phe Arg Thr Val Phe Ser Glu Glu Lys	652
155                             160                             165	
CTG GTT TTC TCT CTG CGC CTG ATG GAG GAG AAC TGG AGC GCC GAG AAG Leu Val Phe Ser Leu Arg Leu Met Glu Glu Asn Trp Ser Ala Glu Lys	700
170                             175                             180	
ATG ACG CCC ACC TTC CAG CTG GGA GAC AGA GCC CAC CTC CAG GCC CAA Met Thr Pro Thr Phe Gln Leu Gly Asp Arg Ala His Leu Gln Ala Gln	748
185                             190                             195	
GTG CAC ACT GGC AGC CAC GTG CCC CTG CGG CTG TTC GTG GAC CAC TGC Val His Thr Gly Ser His Val Pro Leu Arg Leu Phe Val Asp His Cys	796
205                             210                             215	

- 113 -

GTG GCC AGC CTG ACG CCA GAC TGG AGC ACC TCC CCT TAC CAC ACC ATC Val Ala Ser Leu Thr Pro Asp Trp Ser Thr Ser Pro Tyr His Thr Ile 220 225 230	844
GTG GAC TTC CAT GGT TGT CTC GTC GAT GGT CTC ACC GAT GCC TCC TCT Val Asp Phe His Gly Cys Leu Val Asp Gly Leu Thr Asp Ala Ser Ser 235 240 245	892
GCT TTC AAA GCA CCC AGA CCC AGA CCG GAG ATC CTC CAG TTC ACA GTG Ala Phe Lys Ala Pro Arg Pro Arg Pro Glu Ile Leu Gln Phe Thr Val 250 255 260	940
GAT CTG TTC CGT TTT GCT AAT GAC TCC AGA AAC ATG ATA TAT ATC ACC Asp Val Phe Arg Phe Ala Asn Asp Ser Arg Asn Met Ile Tyr Ile Thr 265 270 275 280	988
TGC CAC CTG AAG GTC ACT CCG GTT GAC CGA GTC CCG GAC CAA CTA AAC Cys His Leu Lys Val Thr Pro Val Asp Arg Val Pro Asp Gln Leu Asn 285 290 295	1036
AAA GCC TGT TCC TTC AGC AAG TCC TCC AAC AGG TGG TCC CCG GTT GAA Lys Ala Cys Ser Phe Ser Lys Ser Ser Asn Arg Trp Ser Pro Val Glu 300 305 310	1084
GGC CCC ACT GAC ATC TGT CGA TGC TGT AGC AAG GGG CGC TGT GGC ATT Gly Pro Thr Asp Ile Cys Arg Cys Cys Ser Lys Gly Arg Cys Gly Ile 315 320 325	1132
TCA GGC CGT TCC ATG AGG CTG TCC CAC CGG GAG GGC AGG CCT GTT CCC Ser Gly Arg Ser Met Arg Leu Ser His Arg Glu Gly Arg Pro Val Pro 330 335 340	1180
CGA AGT CGC AGG CAC GTG AGC GAG GAA GCA GAT GTC ACC GTG GGG CGC Arg Ser Arg Arg His Val Thr Val Glu Ala Asp Val Thr Val Gly Pro 345 350 355 360	1228
TTG ATC TTC CTG AGG AAG ATG AAT GAC CGT GGC GTG GAA GGG CCC ACC Leu Ile Phe Leu Arg Lys Met Asn Asp Arg Gly Val Glu Gly Pro Thr 365 370 375	1276
TCC TCT CCC CCT CTG GTG ATG CTG GGC TTA GGC CTG GCT ACT GTG ATG Ser Ser Pro Leu Val Met Leu Gly Leu Gly Leu Ala Thr Val Met 380 385 390	1324
ACC TTG ACT CTG GCT GCC ATT GTC CTG GGT CTC ACT GGG AGG CTT CGG Thr Leu Thr Leu Ala Ala Ile Val Leu Gly Leu Thr Gly Arg Leu Arg 395 400 405	1372
GCT GCT TCT CAC CCC GTG TGC CCT GTG TCT GCT TCC CAA TAAAAAGAAGA Ala Ala Ser His Pro Val Cys Pro Val Ser Ala Ser Gln 410 415 420	1421
AAAGTAAAAA AAAAAAAA AAGCGGCCGC GAATTC	1457

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 421 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Gly Pro Cys Ser Arg Leu Phe Val Cys Phe Leu Leu Trp Gly Ser

- 114 -

1               5               10               15  
Thr Glu Leu Cys Ser Pro Gln Pro Phe Trp Asp Asp Glu Thr Glu Arg  
20               25               30  
Phe Arg Pro Ser Lys Pro Pro Ala Val Met Val Glu Cys Gln Glu Ala  
35               40               45  
Gln Leu Val Val Thr Val Asp Lys Asp Leu Phe Gly Thr Gly Lys Leu  
50               55               60  
Ile Arg Pro Ala Asp Leu Thr Leu Gly Pro Asp Asn Cys Glu Pro Leu  
65               70               75               80  
Ala Ser Ala Asp Thr Asp Gly Val Val Arg Phe Ala Val Gly Leu His  
85               90               95  
Glu Cys Gly Asn Ile Leu Gln Val Thr Asp Asn Ala Leu Val Tyr Ser  
100              105              110  
Thr Phe Leu Leu His Asn Pro Arg Pro Ala Gly Asn Leu Ser Ile Leu  
115              120              125  
Arg Thr Asn Arg Ala Glu Val Pro Ile Glu Cys His Tyr Pro Arg Gln  
130              135              140  
Gly Asn Val Ser Ser Trp Ala Ile Gln Pro Thr Trp Val Pro Phe Arg  
145              150              155              160  
Thr Thr Val Phe Ser Glu Glu Lys Leu Val Phe Ser Leu Arg Leu Met  
165              170              175  
Glu Glu Asn Trp Ser Ala Glu Lys Met Thr Pro Thr Phe Gln Leu Gly  
180              185              190  
Asp Arg Ala His Leu Gln Ala Gln Val His Thr Gly Ser His Val Pro  
195              200              205  
Leu Arg Leu Phe Val Asp His Cys Val Ala Ser Leu Thr Pro Asp Trp  
210              215              220  
Ser Thr Ser Pro Tyr His Thr Ile Val Asp Phe His Gly Cys Leu Val  
225              230              235              240  
Asp Gly Leu Thr Asp Ala Ser Ser Ala Phe Lys Ala Pro Arg Pro Arg  
245              250              255  
Pro Glu Ile Leu Gln Phe Thr Val Asp Val Phe Arg Phe Ala Asn Asp  
260              265              270  
Ser Arg Asn Met Ile Tyr Ile Thr Cys His Leu Lys Val Thr Pro Val  
275              280              285  
Asp Arg Val Pro Asp Gln Leu Asn Lys Ala Cys Ser Phe Ser Lys Ser  
290              295              300  
Ser Asn Arg Trp Ser Pro Val Glu Gly Pro Thr Asp Ile Cys Arg Cys  
305              310              315              320  
Cys Ser Lys Gly Arg Cys Gly Ile Ser Gly Arg Ser Met Arg Leu Ser  
325              330              335  
His Arg Glu Gly Arg Pro Val Pro Arg Ser Arg Arg His Val Thr Glu  
340              345              350  
Glu Ala Asp Val Thr Val Gly Pro Leu Ile Phe Leu Arg Lys Met Asn  
355              360              365

- 115 -

Asp Arg Gly Val Glu Gly Pro Thr Ser Ser Pro Pro Leu Val Met Leu  
 370 375 380

Gly Leu Gly Leu Ala Thr Val Met Thr Leu Thr Leu Ala Ala Ile Val  
 385 390 395 400

Leu Gly Leu Thr Gly Arg Leu Arg Ala Ala Ser His Pro Val Cys Pro  
 405 410 415

Val Ser Ala Ser Gln  
 420

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 125 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AGTTCTGTGCT TATCTGAACA TGTCTTGAGG GATTAGTATG TGTGCTCATT TGGGTTCTTT	60
--------------------------------------------------------------------	----

CCGCTGTATG CTAGGCGTAT CTAGATGCAT TAGCTTGTAA ACACCTCATG TGGACTAAAA	120
-------------------------------------------------------------------	-----

GATGT	125
-------	-----

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 111 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CAGGCCGTAGG CTTGGACTGA AGTTCAAAAG CATTGGCCCC TTCTGATAGC ATACGTTGA	60
-------------------------------------------------------------------	----

AATGTCATTTG TAGTTGCGATG GCTGTATAAG CCAGTCTCAT AGATAAGGGA A	111
------------------------------------------------------------	-----

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 96 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGGGTCCGTC ATGTGATGCT CGCGTATAGTA CGATTTGAA TGCATTATGC GAAATTATTC	60
-------------------------------------------------------------------	----

TAACGACCCG CGATATGGAG GTTGGATTAA GTTACA	96
-----------------------------------------	----

- 116 -

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATGGARAGRT GYCAMGARG

19

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GATCTAAGGA AGATCTATGG ATCC

24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GATCTAAGGA GGTTGTATGG ATCC

24

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 55 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GATCTATGAC CATGATTACG GATTGCCATA GCCGTCGTCC TGCAGCGTCC CGACT

55

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- 117 -

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGGAAACCC GGGCGTTACC CAACTTAATC GATTAGCAGC ACATCCCCCT TCGCCAG

57

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 54 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTCCCAGT CGCGCTGCAG AACGACGGCT AGCGAACATCCG TAATCATGGT CATA

54

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 52 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CTGGCCAAG GGGGATGTG CTGCTAATCG ATTAAAGTTGG GTAACGCCCG GG

52

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 120 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GATCTATGAC CATGATTACG GATTGGCTAG CCGTCGTTCT GCAGCGTCGC GACTGGAAA

60

ATACTGGTAC TAATGCTAA GCGATCGGCA GCAAGACGTC GGAGCGCTGAC CCTTTACCC

120

GGGCGTTACC CAACTTAATC GATTAGCAGC ACATCCCCCT TTGCGCCAGTGG GCCCGCAAT

180

CCCTTGAAATT AGCAAATCGT CGTGTAGGGG GAAAGCGGTC

120

- 118 -

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCGAAGCTTC CGACACCCATC GAACGGCGC

29

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCGCACAAATG TGCCTAACATGA GTGAGCTAAC

30

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CGCGGATCCG GACCAAGGCC AGCGCTTG

28

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 58 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GCGGTGACT CATTAATGAT GATGATGATG ATGCGGGCTC GAGGTGGACC CTTCCACC

58

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- 119 -

- (A) LENGTH: 1701 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1698

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ATG TGG CTG CTG CGG TGC GTT TTG CTG TGT GTC TCA TTA TCT CTT GCT Met Trp Leu Leu Arg Cys Val Leu Leu Cys Val Ser Leu Ser Leu Ala 1 5 10 15	48
GTG ACT GGC CAG CAT AAG CCT GAG GCA CCA GAT TAT TCC AGT GTG CTC Val Ser Gly Gln His Lys Pro Glu Ala Pro Asp Tyr Ser Ser Val Leu 20 25 30	96
CAC TGT GGG CCG TGG AGC TTC CAG TTT GCT GTA AAC CTC AAC CAG GAG His Cys Gly Pro Trp-Ser Phe Gln Phe Ala Val Asn Leu Asn Gln Glu 35 40 45	144
GCA ACG TCT CCT CCT GTA CTA ATA GCT TGG GAC AAC CAA GGG CTG CTG Ala Thr Ser Pro Pro Val Leu Ile Ala Trp Asp Asn Gln Gly Leu Leu 50 55 60	192
CAC GAG CTG CAG AAT GAC TCC GAC TGT GGC ACC TGG ATA AGA AAA GGT His Glu Leu Gln Asn Asp Ser Asp Cys Gly Thr Trp Ile Arg Lys Gly 65 70 75 80	240
CCA GGC AGC TCC GTG CTG TTG GAG GCA ACC TAT AGC AGC TGC TAT GTC Pro Gly Ser Ser Val Val Leu Glu Ala Thr Tyr Ser Ser Cys Tyr Val 85 90 95	288
ACT GAG TGG GTC ACT ATG ACC CAA TGG CCA GGG AGA CTG TGT GAA GCG Thr Glu Trp Val Ser Met Thr Gln Trp Pro Gly Arg Leu Cys Glu Ala 100 105 110	336
CCT CAT GCT ACC ATC CAG GCT GAC CCC CRA GGC CTG TCT CTC CAG GAC Pro His Ala Thr Ile Gln Ala Asp Pro Gln Gly Leu Ser Leu Gln Asp 115 120 125	384
TCC CAC TAC ATC ATG CCA GTT GGA GTT GAA GGA GCA GGC GCG GCT GAA Ser His Tyr Ile Met Pro Val Gly Val Glu Gly Ala Gly Ala Ala Glu 130 135 140	432
CAC AAG GTG GTT ACA GAG AGG AAG CTG CTC AAG TGT CCT ATG GAT CTT His Lys Val Val Thr Glu Arg Lys Leu Leu Lys Cys Pro Met Asp Leu 145 150 155 160	480
CTA GAT GCT CCA GAT ACT GAC TGG TGT GAC TCC ATC CCA GCA CGG GAC Leu Asp Ala Pro Asp Thr Asp Trp Cys Asp Ser Ile Pro Ala Arg Asp 165 170 175	528
AGA CTG CCA TGT GCA CCT TCA CCC ATC TCT CGA GGA GAC TGT GAA GGG Arg Leu Pro Cys Ala Pro Ser Pro Ile Ser Arg Gly Asp Cys Glu Gly 180 185 190	576
CTA GGC TGT TGT TAT AGC TCT GAA GAG GTG AAT TCC TGC TAC TAT GGA Leu Gly Cys Cys Tyr Ser Ser Glu Glu Val Asn Ser Cys Tyr Tyr Gly 195 200 205	624

- 120 -

AAC ACT GTG ACC TTG CAT TGT ACC CGA GAG GGC CAT TTC TCT ATT CCT Asn Thr Val Thr Leu His Cys Thr Arg Glu Gly His Phe Ser Ile Ala 210	215	220	672
GTG TCT CCG AAC GTG ACC TCG CCA CCA CTG CTC TTG GAT TCT GTG CGC Val Ser Arg Asn Val Thr Ser Pro Pro Leu Leu Asp Ser Val Arg 225	230	235	240
TTG GCC CTT AGG AAT GAC AGT GCC TGT AAC CCT GTG ATG GCA ACA CAA Leu Ala Leu Arg Asn Asp Ser Ala Cys Asn Pro Val Met Ala Thr Gln 245	250	255	768
GCT TTT GTT CTG TTC CAG TTT CCA TTT ACT TCC TGT GGC ACC ACA AGA Ala Phe Val Leu Phe Gln Phe Pro Phe Thr Ser Cys Gly Thr Thr Arg 260	265	270	816
CAG ATC ACT GGA GAC CGA GCA GTA TAT GAA AAT GAA CTG GTG GCA ACT Gln Ile Thr Gly Asp Arg Ala Val Tyr Glu Asn Gln Leu Val Ala Thr 275	280	285	864
AGG GAT GTG AAA AAT GGG AGC CGT GGC TCT GTC ACT CGT GAC AGC ATC Arg Asp Val Lys Asn Gly Ser Arg Gly Ser Val Thr Arg Asp Ser Ile 290	295	300	912
TTC AGG CTC CAT GTC ACC TCC ACC TAC TCA GTC ACT ACC AAC TCT CTC Phe Arg Leu His Val Ser Cys Ser Tyr Ser Val Ser Ser Asn Ser Leu 305	310	315	960
CCA ATC AAT GTC CAG GTT TTC ACT CTC CCA CCA CCC TTT CCT GAG ACC Pro Ile Asn Val Gln Val Phe Thr Leu Pro Pro Pro Phe Pro Glu Thr 325	330	335	1008
CAG CCT GGA CCC CTC ACT CTG GAA CCT CAG ATT CCC AAA GAT AAA AAC Gln Pro Gly Pro Leu Thr Leu Gln Leu Gln Ile Ala Lys Asp Lys Asn 340	345	350	1056
TAT GGC TCT TAC TAC GGT GTT GGT GAC TAC CCA GTG GTG AAG TTG CTT Tyr Gly Ser Tyr Tyr Gly Val Gly Asp Tyr Pro Val Val Lys Leu Leu 355	360	365	1104
CGG GAT CCC ATT TAC GTG GAG GTC TCC ATC CTT CAC AGA ACA GAC CCC Arg Asp Pro Ile Tyr Val Glu Val Ser Ile Leu His Arg Thr Asp Pro 370	375	380	1152
TAC CTG GGG CTG CTC CTA CAA CAG TGT TGG GCA ACA CCC AGC ACT GAC Tyr Leu Gly Leu Leu Leu Gln Gln Cys Trp Ala Thr Pro Ser Thr Asp 385	390	395	1200
CCC CTG ACT CAG CCA CAG TGG CCC ATC CTG GTC AAG GGC TGC CCC TAC Pro Leu Ser Gln Pro Gln Trp Pro Ile Leu Val Lys Gly Cys Pro Tyr 405	410	415	1248
ATT GGA GAC AAC TAT CAG ACC CAG CTG ATC CCT GTC CAG AAA GCC TTG Ile Gly Asp Asn Tyr Gln Thr Gln Leu Ile Pro Val Gln Lys Ala Leu 420	425	430	1296
GAT CTT CCA TTT CCC TCT CAC CAC CAG CGC TTC ACC ATC TTC ACC TTC Asp Leu Pro Phe Pro Ser His His Gln Arg Phe Ser Ile Phe Thr Phe 435	440	445	1344
AGC TTT GTG AAC CCT ACA GTG GAG AAA CAG GCC CTC AGG GGA CCG GTG Ser Phe Val Asn Pro Thr Val Glu Lys Gln Ala Leu Arg Gly Pro Val 450	455	460	1392
CAT CTG CAC TCC AGC GTG TCA GTC CAG CCT GCT GAG ACA CCA TCC His Leu His Cys Ser Val Ser Val Cys Gln Pro Ala Glu Thr Pro Ser 465	470	475	1440

- 121 -

TGT GTG CTG ACC TGT CCT GAT CTC AGT CGA AGA AGA AAT TTT GAC AAC Cys Val Val Thr Cys Pro Asp Leu Ser Arg Arg Arg Asn Phe Asp Asn 485 490 495	1488
AGT TCT CAG AAC ACT ACT GCT AGT GTT TCT AGC AAA GGC CCC ATG ATT Ser Ser Gln Asn Thr Thr Ala Ser Val Ser Ser Lys Gly Pro Met Ile 500 505 510	1536
CTA CTC CAA GCC ACT AAG GAC CCT CCA GAA AAG CTC CGT GTT CCT GTA Leu Leu Gln Ala Thr Lys Asp Pro Pro Glu Lys Leu Arg Val Pro Val 515 520 525	1584
GAC TCG AAA GTT CTG TGG GTG GCA GGC CTT TCT GGG ACC TTA ATC CTT Asp Ser Lys Val Leu Trp Val Ala Gly Leu Ser Gly Thr Leu Ile Leu 530 535 540	1632
GGA GCC TTC TTA GTA TCC TAC TTG GCT GTC AAG AAA CAG AAC AGT TGC Gly Ala Leu Leu Val Ser Tyr Leu Ala Val Lys Lys Gln Lys Ser Cys 545 550 555 560	1680
CCA GAC CAA ATG TGT CAA TAA Pro Asp Gln Met Cys Gln 565	1701

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 566 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

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Met Trp Leu Leu Arg Cys Val Leu Leu Cys Val Ser Leu Ser Leu Ala
1           5           10          15

Val Ser Gly Gln His Lys Pro Glu Ala Pro Asp Tyr Ser Ser Val Leu
20          25          30

His Cys Gly Pro Trp Ser Phe Gln Phe Ala Val Asn Leu Asn Gln Glu
35          40          45

Ala Thr Ser Pro Pro Val Leu Ile Ala Trp Asp Asn Gln Gly Leu Leu
50          55          60

His Glu Leu Gln Asn Asp Ser Asp Cys Gly Thr Trp Ile Arg Lys Gly
65          70          75          80

Pro Gly Ser Ser Val Val Leu Ala Thr Tyr Ser Ser Cys Tyr Val
85          90          95

Thr Glu Trp Val Ser Met Thr Gln Trp Pro Gly Arg Leu Cys Glu Ala
100         105         110

Pro His Ala Thr Ile Gln Ala Asp Pro Gln Gly Leu Ser Leu Gln Asp
115         120         125

Ser His Tyr Ile Met Pro Val Gly Val Glu Gly Ala Gly Ala Ala Glu
130         135         140

His Lys Val Val Thr Glu Arg Lys Leu Leu Lys Cys Pro Met Asp Leu
145         150         155         160

Leu Asp Ala Pro Asp Thr Asp Trp Cys Asp Ser Ile Pro Ala Arg Asp

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- 122 -

165	170	175
Arg Leu Pro Cys Ala Pro Ser Pro Ile Ser Arg Gly Asp Cys Glu Gly 180	185	190
Leu Gly Cys Cys Tyr Ser Ser Glu Glu Val Asn Ser Cys Tyr Tyr Gly 195	200	205
Asn Thr Val Thr Leu His Cys Thr Arg Glu Gly His Phe Ser Ile Ala 210	215	220
Val Ser Arg Asn Val Thr Ser Pro Pro Leu Leu Leu Asp Ser Val Arg 225	230	235
Leu Ala Leu Arg Asn Asp Ser Ala Cys Asn Pro Val Met Ala Thr Gln 245	250	255
Ala Phe Val Leu Phe Gln Phe Pro Phe Thr Ser Cys Gly Thr Thr Arg 260	265	270
Gln Ile Thr Gly Asp Arg Ala Val Tyr Glu Asn Glu Leu Val Ala Thr 275	280	285
Arg Asp Val Lys Asn Gly Ser Arg Gly Ser Val Thr Arg Asp Ser Ile 290	295	300
Phe Arg Leu His Val Ser Cys Ser Tyr Ser Val Ser Ser Asn Ser Leu 305	310	315
Pro Ile Asn Val Gln Val Phe Thr Leu Pro Pro Pro Phe Pro Glu Thr 325	330	335
Gln Pro Gly Pro Leu Thr Leu Glu Leu Gln Ile Ala Lys Asp Lys Asn 340	345	350
Tyr Gly Ser Tyr Tyr Gly Val Gly Asp Tyr Pro Val Val Lys Leu Leu 355	360	365
Arg Asp Pro Ile Tyr Val Glu Val Ser Ile Leu His Arg Thr Asp Pro 370	375	380
Tyr Leu Gly Leu Leu Gln Gln Cys Trp Ala Thr Pro Ser Thr Asp 385	390	395
Pro Leu Ser Gln Pro Gln Trp Pro Ile Leu Val Lys Gly Cys Pro Tyr 405	410	415
Ile Gly Asp Asn Tyr Gln Thr Gln Leu Ile Pro Val Gln Lys Ala Leu 420	425	430
Asp Leu Pro Phe Pro Ser His His Gln Arg Phe Ser Ile Phe Thr Phe 435	440	445
Ser Phe Val Asn Pro Thr Val Glu Lys Gln Ala Leu Arg Gly Pro Val 450	455	460
His Leu His Cys Ser Val Ser Val Cys Gln Pro Ala Glu Thr Pro Ser 465	470	475
Cys Val Val Thr Cys Pro Asp Leu Ser Arg Arg Arg Asn Phe Asp Asn 485	490	495
Ser Ser Gln Asn Thr Thr Ala Ser Val Ser Ser Lys Gly Pro Met Ile 500	505	510
Leu Leu Gln Ala Thr Lys Asp Pro Pro Glu Lys Leu Arg Val Pro Val 515	520	525

- 123 -

Asp Ser Lys Val Leu Trp Val Ala Gly Leu Ser Gly Thr Leu Ile Leu  
530 535 540

Gly Ala Leu Leu Val Ser Tyr Leu Ala Val Lys Lys Gln Lys Ser Cys  
545 550 555 560

Pro Asp Gln Met Cys Gln  
565

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2266 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..2235

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ATG GCG TGC AGG CAG AGA GGA GGC TCT TGG AGT CCC TCA GGC TGG TTC	48
Met Ala Cys Arg Gln Arg Gly Gly Ser Trp Ser Pro Ser Gly Trp Phe	
1 5 10 15	
AAT GCA GGC TGG AGC ACC TAC AGG TCG ATT TCT CTC TTC TTG GCC CTT	96
Asn Ala Gly Trp Ser Thr Tyr Arg Ser Ile Ser Leu Phe Phe Ala Leu	
20 25 30	
GTG ACT TCA GGG AAC TCC ATA GAT GTT TCT CAG TTG GTA AAT CCT GCC	144
Val Thr Ser Gly Asn Ser Ile Asp Val Ser Gln Leu Val Asn Pro Ala	
35 40 45	
TTT CCA GGC ACT GTC ACT TGC GAT GAA AGG GAA ATA ACA GTG GAG TTC	192
Phe Pro Gly Thr Val Thr Cys Asp Glu Arg Glu Ile Thr Val Glu Phe	
50 55 60	
CCA AGC AGT CCT GGC ACC AAG AAA TGG CAT GCA TCT GTG GTG GAT CCT	240
Pro Ser Ser Pro Gly Thr Lys Lys Trp His Ala Ser Val Val Asp Pro	
65 70 75 80	
CTT GGT CTC GAC ATG CCG AAC TGC ACT TAC ATC CTG GAC CCA GAA AAG	288
Leu Gly Leu Asp Met Pro Asn Cys Thr Tyr Ile Leu Asp Pro Glu Lys	
85 90 95	
CTC ACC CTG AGG GCT ACC TAT GAT AAC TGT ACC AGG AGA GTG CAT GGT	336
Leu Thr Leu Arg Ala Thr Tyr Asp Asn Cys Thr Arg Arg Val His Gly	
100 105 110	
GGA CRC CAG ATG ACC ATC AGA GTC ATG AAC AAC AGT GCT GCC TTA AGA	384
Gly His Gln Met Thr Ile Arg Val Met Asn Asn Ser Ala Ala Leu Arg	
115 120 125	
CAC GGA GCT GTC ATG TAT CAG TTC TGT CCA GCT ATG CAA GTA GAA	432
His Gly Ala Val Met Tyr Gln Phe Phe Cys Pro Ala Met Gln Val Glu	
130 135 140	
GAG ACC CAG GGG CTT TCA GCA TCT ACA ATC TGC CAG AAG GAT TTC ATG	480
Glu Thr Gln Gly Leu Ser Ala Ser Thr Ile Cys Gln Lys Asp Phe Met	
145 150 155 160	

- 124 -

TCT TTT TCC TTG CCA CGG GTC TTC TCT GGC TTG GCT GAC GAC AGT AAG Ser Phe Ser Leu Pro Arg Val Phe Ser Gly Leu Ala Asp Asp Ser Lys 165	170	175	528
GGG ACC AAA GTT CAG ATG CGA TGG AGC ATT GAG GTT GGT GAT GGT GCA Gly Thr Lys Val Gln Met Gly Trp Ser Ile Glu Val Gly Asp Gly Ala 180	185	190	576
AGA GCC AAA ACT CTG ACC CTG CCA GAG GCC ATG AAG GAA GGC TTC AGC Arg Ala Lys Thr Leu Thr Leu Pro Glu Ala Met Lys Glu Gly Phe Ser 195	200	205	624
CTC TTG ATT GAC AAC CAC AGG ATG ACC TTC CAT GTG CCA TTC AAT GCC Leu Leu Ile Asp Asn His Arg Met Thr Phe His Val Pro Phe Asn Ala 210	215	220	672
ACT GGA GTG ACT CAC TAT GTG CAA GGT AAC AGT CAT CTC TAC ATG GTG Thr Gly Val Thr His Tyr Val Gln Gly Asn Ser His Leu Tyr Met Val 225	230	235	720
TCT CTG AAG CTT ACA TTT ATA TCT CCT GGA CAG AAG GTG ATC TTC TCT Ser Leu Lys Leu Thr Phe Ile Ser Pro Gly Gln Lys Val Ile Phe Ser 245	250	255	768
TCA CAA GCT ATT TTG GCA CCA GAT CCT GTG ACC TGC RAT GCC ACA CAC Ser Gln Ala Ile Cys Ala Pro Asp Pro Val Thr Cys Asn Ala Thr His 260	265	270	816
ATG ACT CTC ACC ATA CCA GAG TTT CCT GGG AAG CTT AAG TCT GTG AGC Met Thr Leu Thr Ile Pro Glu Phe Pro Gly Lys Leu Lys Ser Val Ser 275	280	285	864
TTT GAA AAC CAG AAC ATT GAT GTG AGC CAG CTG CAT GAC AAT GGA ATT Phe Glu Asn Gln Asn Ile Asp Val Ser Gln Leu His Asp Asn Gly Ile 290	295	300	912
GAT CTA GAA GCA ACA AAT GGC ATG AAA TTG CAT TTC AGC AAA ACT CTC Asp Leu Glu Ala Thr Asn Gly Met Lys Leu His Phe Ser Lys Thr Leu 305	310	315	960
CTC AAA ACG AAA TTA TCT GAA AAA TGC CTA CTC CAT CAG TTC TAC TTA Leu Lys Thr Lys Leu Ser Glu Lys Cys Leu Leu His Gln Phe Tyr Leu 325	330	335	1008
GCT TCA CTC AAG CTG ACC TTT CTC CTT CGG CCA GAG ACA GTA TCC ATG Ala Ser Leu Lys Leu Thr Phe Leu Leu Arg Pro Glu Thr Val Ser Met 340	345	350	1056
GTG ATC TAT CCT GAG TGT CTC TGT GAG TCA CCC GTT TCT ATA GTT ACA Val Ile Tyr Pro Glu Cys Leu Cys Glu Ser Pro Val Ser Ile Val Thr 355	360	365	1104
GGG GAG CTG TGC ACC CAG GAT GGG TTT ATG GAC GTC GAG GTC TAC AGC Gly Glu Leu Cys Thr Gln Asp Gly Phe Met Asp Val Glu Val Tyr Ser 370	375	380	1152
TAC CAA ACA CAA CCA GCT CTT GAC CTG GGT ACT CTG AGG GTG GGA AAC Tyr Gln Thr Gln Pro Ala Leu Asp Leu Gly Thr Leu Arg Val Gly Asn 385	390	395	1200
TCA TCC TGC CAG CCT GTC TTT GAG GCT CAG TCT CAG GGG CTG GTA CGG Ser Ser Cys Gln Pro Val Phe Glu Ala Gln Ser Gln Gly Leu Val Arg 405	410	415	1248
TTC CAC ATA CCC CCT CTC AAT GGA TGT GGA AGC AGA TAT AAG TTC GAA GAT Phe His Ile Pro Leu Asn Gly Cys Gly Thr Arg Tyr Lys Phe Glu Asp 420	425	430	1296

- 125 -

GAT AAA GTC GTC TAT GAA AAC GAA ATA CAT GCT CTC TGG AGC GAT TTT	1344
Asp Lys Val Val Tyr Glu Asn Glu Ile His Ala Leu Trp Thr Asp Phe	
435 440 445	
CCT CCA AGC AAA ATA TCT AGA GAC AGT GAG TTC AGA ATG ACA GTG AAG	1392
Pro Pro Ser Lys Ile Ser Arg Asp Ser Glu Phe Arg Met Thr Val Lys	
450 455 460	
TCT TCT TAT AGC AGG AAT GAC ATG CTA CTA AAC ATC AAC GTT GAA AGC	1440
Cys Ser Tyr Ser Arg Asn Asp Met Leu Leu Asn Ile Asn Val Glu Ser	
465 470 475 480	
CTT ACT CCT CCA GTC GGC TCA GTG AAG TTG GGT CCA TTT ACC TTG ATC	1488
Leu Thr Pro Pro Val Ala Ser Val Lys Leu Gly Pro Phe Thr Leu Ile	
485 490 495	
CTG CAA AGC TAC CCA GAT AAT TCC TAC CAA CAA CCT TAT TGG GAA AAC	1536
Leu Gln Ser Tyr Pro Asp Asn Ser Tyr Gln Gln Pro Tyr Gly Glu Asn	
500 505 510	
GAG TAC CCT CTA GTG AGA TTC CTC CGC CAA CCA ATT TAC AIG GAA GTG	1584
Glu Tyr Pro Leu Val Arg Phe Leu Arg Gln Pro Ile Tyr Met Glu Val	
515 520 525	
AGA GTC CTA AAC AGG GAT GAC CCC AAC ATC AAG CTG GTC TTA GAT GAC	1632
Arg Val Leu Asn Arg Asp Asp Pro Asn Ile Lys Leu Val Leu Asp Asp	
530 535 540	
TGG TCG GCG AGC TCC ACC ATG GAT CCA GAC TCT TTC CCC CAG TGG AAC	1680
Cys Trp Ala Thr Ser Thr Met Asp Pro Asp Ser Phe Pro Gln Trp Asn	
545 550 555 560	
GTT GTC GTG GAT GGC TGT GCA TAT GAC CTG GAC AAC TAC CAG ACC ACC	1728
Val Val Val Asp Gly Cys Ala Tyr Asp Leu Asp Asn Tyr Gln Thr Thr	
565 570 575	
TTC CAT CCA GTC GGC TCC TCT GTG ACC CAT CCT GAT CAC TAT CAG AGG	1776
Phe His Pro Val Gly Ser Ser Val Val Thr His Pro Asp His Tyr Gln Arg	
580 585 590	
TTT GAC ATG AAG GCT TTT GCC TTT GTC TCA GAA GCC CAC GTG CTC TCT	1824
Phe Asp Met Lys Ala Phe Ala Phe Val Ser Glu Ala His Val Leu Ser	
595 600 605	
AGC CTC GTC TAC TTC CAC TGC AGT GCC TTA ATC TGT ATT CGA CTC TCC	1872
Ser Leu Val Tyr Phe His Cys Ser Ala Leu Ile Cys Asn Arg Leu Ser	
610 615 620	
CCT GAC TCC CCA CTG TGT TCT GTG ACC TGC CCT GTG TCC TCT AGG CAC	1920
Pro Asp Ser Pro Leu Cys Ser Val Thr Cys Pro Val Ser Ser Arg His	
625 630 635 640	
AGG CGA GCC ACA GGG GCC ACT GAA GCA GAG AAA ATG ACA GTC AGC CTC	1968
Arg Arg Ala Thr Gly Ala Thr Glu Ala Glu Lys Met Thr Val Ser Leu	
645 650 655	
CCA GGA CCC ATT CTC CTG TTG TCA GAT GAC TCC TCA TTC AGA GGT GTC	2016
Pro Gly Pro Ile Leu Leu Leu Ser Asp Asp Ser Ser Phe Arg Gly Val	
660 665 670	
GGC TCA TCT GAT CTA AAA GCA ACT GGG AGC AGT GGG GAG AAG AGT AGG	2064
Gly Ser Ser Asp Leu Lys Ala Ser Gly Ser Ser Gly Glu Lys Ser Arg	
675 680 685	
AGT GAA ACA GGG GAG GAG GTT GGC TCA CGA GGT GCT ATG GAC ACC AAA	2112
Ser Glu Thr Gly Glu Val Gly Ser Arg Gly Ala Met Asp Thr Lys	
690 695 700	

- 126 -

GGG CAC AAG ACT GCT GGA GAT GTT GGT TCC AAA GCT GTG GCT GCT GTG Gly His Lys Thr Ala Gly Asp Val Gly Ser Lys Ala Val Ala Ala Val 705 710 715 720	2160
GCT GCC TTT GCA GGT GTG GTG GCA ACT CTA GGC TTC ATC TAC TAC CTG Ala Ala Phe Ala Gly Val Val Ala Thr Leu Gly Phe Ile Tyr Tyr Leu 725 730 735	2208
TAC GAG AAA AGG ACT GTG TCA AAC CAC TAAATGGGCT TCTAAATAAA Tyr Glu Lys Arg Thr Val Ser Asn His 740 745	2255
GCAGTCAAAAA T	2266

## (2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 745 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Ala Cys Arg Gln Arg Gly Gly Ser Trp Ser Pro Ser Gly Trp Phe 1 5 10 15
Asn Ala Gly Trp Ser Thr Tyr Arg Ser Ile Ser Leu Phe Phe Ala Leu 20 25 30
Val Thr Ser Gly Asn Ser Ile Asp Val Ser Gln Leu Val Asn Pro Ala 35 40 45
Phe Pro Gly Thr Val Thr Cys Asp Glu Arg Glu Ile Thr Val Glu Phe 50 55 60
Pro Ser Ser Pro Gly Thr Lys Lys Trp His Ala Ser Val Val Asp Pro 65 70 75 80
Leu Gly Leu Asp Met Pro Asn Cys Thr Tyr Ile Leu Asp Pro Glu Lys 85 90 95
Leu Thr Leu Arg Ala Thr Tyr Asp Asn Cys Thr Arg Arg Val His Gly 100 105 110
Gly His Gln Met Thr Ile Arg Val Met Asn Asn Ser Ala Ala Leu Arg 115 120 125
His Gly Ala Val Met Tyr Gln Phe Phe Cys Pro Ala Met Gln Val Glu 130 135 140
Glu Thr Gln Gly Leu Ser Ala Ser Thr Ile Cys Gln Lys Asp Phe Met 145 150 155 160
Ser Phe Ser Leu Pro Arg Val Phe Ser Gly Leu Ala Asp Asp Ser Lys 165 170 175
Gly Thr Lys Val Gln Met Gly Trp Ser Ile Glu Val Gly Asp Gly Ala 180 185 190
Arg Ala Lys Thr Leu Thr Leu Pro Glu Ala Met Lys Glu Gly Phe Ser 195 200 205
Leu Leu Ile Asp Asn His Arg Met Thr Phe His Val Pro Phe Asn Ala 210 215 220

- 127 -

Thr Gly Val Thr His Tyr Val Gln Gly Asn Ser His Leu Tyr Met Val  
 225 230 235 240  
 Ser Leu Lys Leu Thr Phe Ile Ser Pro Gly Gln Lys Val Ile Phe Ser  
 245 250 255  
 Ser Gln Ala Ile Cys Ala Pro Asp Pro Val Thr Cys Asn Ala Thr His  
 260 265 270  
 Met Thr Leu Thr Ile Pro Glu Phe Pro Gly Lys Leu Lys Ser Val Ser  
 275 280 285  
 Phe Glu Asn Gln Asn Ile Asp Val Ser Gln Leu His Asp Asn Gly Ile  
 290 295 300  
 Asp Leu Glu Ala Thr Asn Gly Met Lys Leu His Phe Ser Lys Thr Leu  
 305 310 315 320  
 Leu Lys Thr Lys Leu Ser Glu Lys Cys Leu Leu His Gln Phe Tyr Leu  
 325 330 335  
 Ala Ser Leu Lys Leu Thr Phe Leu Leu Arg Pro Glu Thr Val Ser Met  
 340 345 350  
 Val Ile Tyr Pro Glu Cys Leu Cys Glu Ser Pro Val Ser Ile Val Thr  
 355 360 365  
 Gly Glu Leu Cys Thr Gln Asp Gly Phe Met Asp Val Glu Val Tyr Ser  
 370 375 380  
 Tyr Gln Thr Gln Pro Ala Leu Asp Leu Gly Thr Leu Arg Val Gly Asn  
 385 390 395 400  
 Ser Ser Cys Gln Pro Val Phe Glu Ala Gln Ser Gln Gly Leu Val Arg  
 405 410 415  
 Phe His Ile Pro Leu Asn Gly Cys Gly Thr Arg Tyr Lys Phe Glu Asp  
 420 425 430  
 Asp Lys Val Val Tyr Glu Asn Glu Ile His Ala Leu Trp Thr Asp Phe  
 435 440 445  
 Pro Pro Ser Lys Ile Ser Arg Asp Ser Glu Phe Arg Met Thr Val Lys  
 450 455  
 Cys Ser Tyr Ser Arg Asn Asp Met Leu Leu Asn Ile Asn Val Glu Ser  
 465 470 475 480  
 Leu Thr Pro Pro Val Ala Ser Val Lys Leu Gly Pro Phe Thr Leu Ile  
 485 490 495  
 Leu Gln Ser Tyr Pro Asp Asn Ser Tyr Gln Gln Pro Tyr Gly Glu Asn  
 500 505 510  
 Glu Tyr Pro Leu Val Arg Phe Leu Arg Gln Pro Ile Tyr Met Glu Val  
 515 520 525  
 Arg Val Leu Asn Arg Asp Asp Pro Asn Ile Lys Leu Val Leu Asp Asp  
 530 535  
 Cys Trp Ala Thr Ser Thr Met Asp Pro Asp Ser Phe Pro Gln Trp Asn  
 545 550 555 560  
 Val Val Val Asp Gly Cys Ala Tyr Asp Leu Asp Asn Tyr Gln Thr Thr  
 565 570 575  
 Phe His Pro Val Gly Ser Ser Val Thr His Pro Asp His Tyr Gln Arg

- 128 -

580	585	590
Phe Asp Met Lys Ala Phe Ala Phe Val Ser Glu Ala His Val Leu Ser		
595	600	605
Ser Leu Val Tyr Phe His Cys Ser Ala Leu Ile Cys Asn Arg Leu Ser		
610	615	620
Pro Asp Ser Pro Leu Cys Ser Val Thr Cys Pro Val Ser Ser Arg His		
625	630	635
Arg Arg Ala Thr Gly Ala Thr Glu Ala Glu Lys Met Thr Val Ser Leu		
645	650	655
Pro Gly Pro Ile Leu Leu Leu Ser Asp Asp Ser Ser Phe Arg Gly Val		
660	665	670
Gly Ser Ser Asp Leu Lys Ala Ser Gly Ser Ser Gly Glu Lys Ser Arg		
675	680	685
Ser Glu Thr Gly Glu Glu Val Gly Ser Arg Gly Ala Met Asp Thr Lys		
690	695	700
Gly His Lys Thr Ala Gly Asp Val Gly Ser Lys Ala Val Ala Ala Val		
705	710	715
Ala Ala Phe Ala Gly Val Val Ala Thr Leu Gly Phe Ile Tyr Tyr Leu		
725	730	735
Tyr Glu Lys Arg Thr Val Ser Asn His		
740	745	

## (2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 560 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 15..506

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GAATTCCGGG CGCG TCC TCT GTG ACC CAT CCT GAT CAC TAT CAG AGG TTT			
Ser Ser Val Thr His Pro Asp His Tyr Gln Arg Phe			
1	5	10	
GAC ATG AAG GCT TTT GCC TTT GTA TCA GAG GCC CAT GTG CTC TCT AGC			
Asp Met Lys Ala Phe Ala Phe Val Ser Glu Ala His Val Leu Ser Ser			
15	20	25	
CTG GTC TAC TTC CAC TGC AGT GCC TTA ATC TGC AAT CGA CTC TCT CCA			
Leu Val Tyr Phe His Cys Ser Ala Leu Ile Cys Asn Arg Leu Ser Pro			
30	35	40	
GAC CCT CCT CTG TGT TCT GTG ACC TGC CCT GTG TCA TCT AGG CAC AGG			
Asp Ser Pro Leu Cys Ser Val Thr Cys Pro Val Ser Ser Arg His Arg			
45	50	55	60
CGA GCC ACA GGG GCC ACT GAA GCA GAG AAA ATG ACA GTC AGC CTC CCA			
242			

- 129 -

Arg Ala Thr Gly Ala Thr Glu Ala Glu Lys Met Thr Val Ser Leu Pro	75		
65			
GGA CCC ATT CTC CTC TTG TCA GAC GAC TCC TCA TTC AGA GGT GTT GGC	290		
Gly Pro Ile Leu Leu Ser Asp Asp Ser Ser Phe Arg Gly Val Gly			
80	85	90	
TCA TCT GAT CTA AAA GCA AGT GGG AGC AGT GGG GAG AAC AGT AGG AGC	338		
Ser Ser Asp Leu Lys Ala Ser Gly Ser Ser Gly Glu Asn Ser Arg Ser			
95	100	105	
GAA ACA GGG GAG GAG GTT GGC TCA CGA GAT GTT ATG GAC ACC AAA GGG	386		
Glu Thr Gly Glu Glu Val Gly Ser Arg Asp Val Met Asp Thr Lys Gly			
110	115	120	
CAC AGG ACT GCT GGA GAT GTT GGT TCC AAA GCT GTG GCT GCT GTG GCT	434		
His Arg Thr Ala Gly Asp Val Gly Ser Lys Ala Val Ala Ala Val Ala			
125	130	135	140
GCC TTG GCA GGT GTG GTG GCA ACT CTA GGC TTC ATC TGT TAC CTG TAT	482		
Ala Leu Ala Gly Val Val Ala Thr Leu Gly Phe Ile Cys Tyr Leu Tyr			
145	150	155	
AAG AAA AGG ACT GTG TCA AAT CAC TAAATGGGCT TCTAAATAAA GCAGTCAAA	536		
Lys Lys Arg Thr Val Ser Asn His			
160			
AAAAAAAAAA CGGGCCCGGA ATTC	560		

## (2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 164 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Ser Ser Val Thr His Pro Asp His Tyr Gln Arg Phe Asp Met Lys Ala			
1	5	10	15
Phe Ala Phe Val Ser Glu Ala His Val Leu Ser Ser Leu Val Tyr Phe			
20	25	30	
His Cys Ser Ala Leu Ile Cys Asn Arg Leu Ser Pro Asp Ser Pro Leu			
35	40	45	
Cys Ser Val Thr Cys Pro Val Ser Ser Arg His Arg Arg Ala Thr Gly			
50	55	60	
Ala Thr Glu Ala Glu Lys Met Thr Val Ser Leu Pro Gly Pro Ile Leu			
65	70	75	80
Leu Leu Ser Asp Asp Ser Ser Phe Arg Gly Val Gly Ser Ser Asp Leu			
85	90	95	
Lys Ala Ser Gly Ser Ser Gly Glu Asn Ser Arg Ser Glu Thr Gly Glu			
100	105	110	
Glu Val Gly Ser Arg Asp Val Met Asp Thr Lys Gly His Arg Thr Ala			
115	120	125	
Gly Asp Val Gly Ser Lys Ala Val Ala Ala Val Ala Leu Ala Gly			
130	135	140	

- 130 -

Val Val Ala Thr Leu Gly Phe Ile Cys Tyr Leu Tyr Lys Lys Arg Thr  
 145                   150                   155                   160

Val Ser Asn His

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 866 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 12..821

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GAATTCGCGG C CGC CGT GGC TCT GTC ACT CGT GAC AGC ATC TTC AGG CTC	50
Arg Arg Gly Ser Val Thr Arg Asp Ser Ile Phe Arg Leu	
1                   5                   10	
CAT GTC AGC TGC AGC TAC TCA GTA AGT AGC AAC TCT CTC CCA ATC AAG	98
His Val Ser Cys Ser Tyr Ser Val Ser Ser Asn Ser Leu Pro Ile Lys	
15                20                25	
GTC CAG GTT TTT ACT CTC CCA CCA CCC TTT CCT GAG ACC CAG CCT GGA	146
Val Gln Val Phe Thr Leu Pro Pro Pro Phe Pro Glu Thr Gln Pro Gly	
30                35                40                45	
CCC CTC ACT CTG GAA CTT CAG ATT GCC AAA GAT AAA AAC TAT GGC TCC	194
Pro Leu Thr Leu Glu Leu Gln Ile Ala Lys Asp Lys Asn Tyr Gly Ser	
50                55                60	
TAC TAT GGT GTT GGT GAC TAC CCC GTG GTG AAG TTG CTT CGG GAT CCC	242
Tyr Tyr Gly Val Gly Asp Tyr Pro Val Val Lys Leu Arg Asp Pro	
65                70                75	
ATC TAT GTG GAG GTC TCC ATC CTT CAC AGA ACA GAC CCC TCC CTG GGG	290
Ile Tyr Val Glu Val Ser Ile Leu His Arg Thr Asp Pro Ser Leu Gly	
80                85                90	
CTG CTC CTA CAT CAG TGT TGG GCA ACA CCC AGC ACA GAC CCA CTG ACT	338
Leu Leu Leu His Gln Cys Trp Ala Thr Pro Ser Thr Asp Pro Leu Pro	
95                100                105	
CAG CCA CAG TGG CCC ATC CTG GTC AAG GGC TGC CCC TAC ATT GGA GAC	386
Gln Pro Gln Trp Pro Ile Leu Val Lys Gly Cys Pro Tyr Ile Gly Asp	
110               115                120                125	
AAC TAT CAG ACC CAG CTG ATC CCT GTC CAG AAA GCC TTG GAT CTT CCA	434
Asn Tyr Gln Thr Gln Leu Ile Pro Val Gln Lys Ala Leu Asp Leu Pro	
130               135                140	
TTT CCC TCT CAC TAC CAG CGC TTC AGC ATC TTC ACC TTC AGC TTT GTG	482
Phe Pro Ser His Tyr Gln Arg Phe Ser Ile Phe Thr Phe Ser Phe Val	
145               150                155	
GAC CCT ACA GCG GAG AAA CAG GCC CTC AGG GGA CGG GTG CAT CTG CAC	530
Asp Pro Thr Ala Glu Lys Gln Ala Leu Arg Gly Pro Val His Leu His	
160               165                170	

- 131 -

TGC AGT GTG TCA GTC TGC CAG CCT GCT GAG ACA CCA TCC TGT GCG GTA Cys Ser Val Ser Val Cys Gln Pro Ala Glu Thr Pro Ser Cys Ala Val 175 180 185	578
ACC TGT CCT GAT CTC AGT CGA AGA AAT TCA GGC ACC ATT TTT CAG AAC Thr Cys Pro Asp Leu Ser Arg Arg Asn Ser Gly Thr Ile Phe Gln Asn 190 195 200 205	626
ACT ACT GCT AGT GTT TCT AGC AAA GGC CCC ATG ATT CTA CTC CAA GCC Thr Thr Ala Ser Val Ser Ser Lys Gly Pro Met Ile Leu Leu Gln Ala 210 215 220	674
ACT AAG GAC CCT CCA GAA AAG CTC CGT GCT CCT GTA GAC TCA AAA GTT Thr Lys Asp Pro Pro Glu Lys Leu Arg Ala Pro Val Asp Ser Lys Val 225 230 235	722
CTG TGG CTG GCA GGC CTT TCT GGG ACC TTA ATC CTT GGA GCC TTA GTA Leu Trp Val Ala Gly Leu Ser Gly Thr Leu Ile Leu Gly Gly Leu Val 240 245 250	770
GTA TCC TAC TTG GCT ATC AAA CAG CTG AAT TGT CCA GAC CAA ACA TGT Val Ser Tyr Leu Ala Ile Lys Gln Leu Asn Cys Pro Asp Gln Thr Cys 255 260 265	818
CAA TAAAACCAAGA CTGTACTCCC AAAAAAAAAA AGCGGCCGCG AATTC Gln 270	866

**(2) INFORMATION FOR SEQ ID NO:47:**

- (i) SEQUENCE CHARACTERISTICS:**
  - (A) LENGTH: 270 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

**(ii) MOLECULE TYPE: protein**

**(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:**

Arg Arg Gly Ser Val Thr Arg Asp Ser Ile Phe Arg Leu His Val Ser 1 5 10 15
Cys Ser Tyr Ser Val Ser Ser Asn Ser Leu Pro Ile Lys Val Gln Val 20 25 30
Phe Thr Leu Pro Pro Pro Phe Pro Glu Thr Gln Pro Gly Pro Leu Thr 35 40 45
Leu Glu Leu Gln Ile Ala Lys Asp Lys Asn Tyr Gly Ser Tyr Tyr Gly 50 55 60
Val Gly Asp Tyr Pro Val Val Lys Leu Leu Arg Asp Pro Ile Tyr Val 65 70 75 80
Glu Val Ser Ile Leu His Arg Thr Asp Pro Ser Leu Gly Leu Leu Leu 85 90 95
His Gln Cys Trp Ala Thr Pro Ser Thr Asp Pro Leu Ser Gln Pro Gln 100 105 110
Trp Pro Ile Leu Val Lys Gly Cys Pro Tyr Ile Gly Asp Asn Tyr Gln 115 120 125
Thr Gln Leu Ile Pro Val Gln Lys Ala Leu Asp Leu Pro Phe Pro Ser 130 135 140

- 132 -

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His Tyr Gln Arg Phe Ser Ile Phe Thr Phe Ser Phe Val Asp Pro Thr
145 150 155 160

Ala Glu Lys Gln Ala Leu Arg Gly Pro Val His Leu His Cys Ser Val
165 170 175

Ser Val Cys Gln Pro Ala Glu Thr Pro Ser Cys Ala Val Thr Cys Pro
180 185 190

Asp Leu Ser Arg Arg Asn Ser Gly Thr Ile Phe Gln Asn Thr Thr Ala
195 200 205

Ser Val Ser Ser Lys Gly Pro Met Ile Leu Leu Gln Ala Thr Lys Asp
210 215 220

Pro Pro Glu Lys Leu Arg Ala Pro Val Asp Ser Lys Val Leu Trp Val
225 230 235 240

Ala Gly Leu Ser Gly Thr Leu Ile Leu Gly Gly Leu Val Val Ser Tyr
245 250 255

Leu Ala Ile Lys Gln Leu Asn Cys Pro Asp Gln Thr Cys Gln
260 265 270

```

(2) INFORMATION FOR SEO ID NO: 48:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 722 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- ii) MOLECULE TYPE: cDNA
- ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 15..682

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GAATTCGCGG	CCGC	ATC	CAC	ACT	GCG	AGC	CAC	GTG	CCA	CTG	CGG	TTG	TTT	50		
Ile	His	Thr	Gly	Ser	His	Val	Pro	Leu	Arg	Leu	Phe					
1				5						10						
GTG	GAC	CAC	TGC	GTG	GCC	ACA	CCA	ACA	GAC	CAG	AAT	GCC	TCC	98		
Val	Asp	His	Cys	Val	Ala	Thr	Pro	Thr	Pro	Asp	Gln	Asn	Ala	Ser	Pro	
15					20					25						
TAT	CAC	ACC	ATC	GTG	GAC	TTC	CAT	GGC	TGT	CTT	GTC	GAT	GGT	CTC	ACT	146
Tyr	His	Thr	Ile	Val	Asp	Phe	His	Gly	Cys	Leu	Val	Asp	Gly	Leu	Thr	
30					35				40							
GAT	GCC	TCT	TCT	GCG	TTC	AAA	GTT	CCT	CGA	CCC	GGG	CCR	GAT	ACA	CTC	194
Asp	Ala	Ser	Ser	Ala	Phe	Lys	Val	Pro	Arg	Pro	Gly	Pro	Asp	Thr	Leu	
45					50				55			60				
CAG	TTC	ACA	GTG	GAT	GTC	TTC	CAC	TTT	GCT	AAT	GAC	TCC	AGA	AAC	ATG	242
Gln	Phe	Thr	Val	Asp	Val	Phe	His	Phe	Ala	Asn	Asp	Ser	Arg	Asn	Met	
					65			70		75						
ATA	TAC	ATC	ACC	TGC	CAC	CTG	AAG	GCC	ATC	CCA	GCT	GAG	CAG	GAA	CCA	290
Ile	Tyr	Ile	Thr	Cys	His	Leu	Lys	Ala	Ile	Pro	Ala	Glu	Gln	Glu	Pro	
80					85			90								
GAC	GAA	CTC	AAC	AAA	GCC	TGT	TCC	TTC	AGC	AAG	TCT	TCC	AAC	AGC	TGG	338

- 133 -

Asp Glu Leu Asn Lys Ala Cys Ser Phe Ser Lys Ser Ser Asn Ser Trp		
95	100	105
TTC CCA GTG GAA GGC CCA GCT GAC ATC TGT CAA TGC TGT AGC AAG GGT		386
Phe Pro Val Glu Gly Pro Ala Asp Ile Cys Gln Cys Cys Ser Lys Gly		
110	115	120
GAC TGT GGC ACT CCA AGC CAT TCC AGG AGG CAG CCC CAT GTC GTG AGC		434
Asp Cys Gly Thr Pro Ser His Ser Arg Arg Gln Pro His Val Val Ser		
125	130	135
CAG TGG TCC AGG TCT TCT CGT AAC CGC AGG CAT GTG ACA GAA GAA		482
Gln Trp Ser Arg Ser Ala Ser Arg Asn Arg Arg His Val Thr Glu Glu		
145	150	155
GCA GAT ATC ACC GTG GGG CCA CTG ATC TTC CTG GAC AGG AGT GCT GAC		530
Ala Asp Ile Thr Val Gly Pro Leu Ile Phe Leu Asp Arg Ser Ala Asp		
160	165	170
TAT GAA GTA GAA CAG TGG GCC TTG CCG ACT GAC ACC TCC CTG CTG CTG		578
Tyr Glu Val Glu Gln Trp Ala Leu Pro Thr Asp Thr Ser Val Leu Leu		
175	180	185
CTG GCC ATA GGC CTG GCC GTG GCA TCT CTG ACT CTG ACC GCT GTT		626
Leu Gly Ile Gly Leu Ala Val Val Ala Ser Leu Thr Leu Thr Ala Val		
190	195	200
ATC CTG ATT TTC ACC AGG AGG TGG CGC ACT GCC TCC CGC CCT GTG TCT		674
Ile Leu Ile Phe Thr Arg Arg Trp Arg Thr Ala Ser Arg Pro Val Ser		
205	210	215
220		
GTG TCC CAA TAAAAAGAAGA AAGCAGTAAA AAAAAGCCGGC CGCGAATTG		722
Val Ser Gln		

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 223 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ile His Thr Gly Ser His Val Pro Leu Arg Leu Phe Val Asp His Cys		
1	5	10
15		
Val Ala Thr Pro Thr Pro Asp Gln Asn Ala Ser Pro Tyr His Thr Ile		
20	25	30
30		
Val Asp Phe His Gly Cys Leu Val Asp Gly Leu Thr Asp Ala Ser Ser		
35	40	45
45		
Ala Phe Lys Val Pro Arg Pro Gly Pro Asp Thr Leu Gln Phe Thr Val		
50	55	60
60		
Asp Val Phe His Phe Ala Asn Asp Ser Arg Asn Met Ile Tyr Ile Thr		
65	70	75
75		
Cys His Leu Lys Ala Ile Pro Ala Glu Gln Glu Pro Asp Glu Leu Asn		
85	90	95
95		
Lys Ala Cys Ser Phe Ser Lys Ser Ser Asn Ser Trp Phe Pro Val Glu		
100	105	110

- 134 -

Gly Pro Ala Asp Ile Cys Gln Cys Cys Ser Lys Gly Asp Cys Gly Thr  
 115 120 125

Pro Ser His Ser Arg Arg Gln Pro His Val Val Ser Gln Trp Ser Arg  
 130 135 140

Ser Ala Ser Arg Asn Arg Arg His Val Thr Glu Glu Ala Asp Ile Thr  
 145 150 155 160

Val Gly Pro Leu Ile Phe Leu Asp Arg Ser Ala Asp Tyr Glu Val Glu  
 165 170 175

Gln Trp Ala Leu Pro Thr Asp Thr Ser Val Leu Leu Leu Gly Ile Gly  
 180 185 190

Leu Ala Val Val Ala Ser Leu Thr Leu Thr Ala Val Ile Leu Ile Phe  
 195 200 205

Thr Arg Arg Trp Arg Thr Ala Ser Arg Pro Val Ser Val Ser Gln  
 210 215 220

## (2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 28 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CGCCCTTCCCC AGCAACTGCA CCATCACCAAC CATGGG

36

## (2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 45 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GATCCCCATG GTGGTGGTGA TGGTCCAGTT GCTGGGAAGG GCGAT

45

## (2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- 135 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GATCCCTCGA GCCACCATCA CCACCATCAT G

31

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AATTCCATGAT GGTGGTGATG GTGGCTCGAG G

31

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CCCGGATCCG CAGACCATCT GGCCAACTGA G

31

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GCGCTCGAGG GCATATGGCT GCCAGTGTG

29

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- 136 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CGCGCTAGCA GATCTATGGC GCCGAGCTGG AGGTTC

36

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 49 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CGCGGATCCT ATTAATGGTG GTGATGGTGG TGACTAGTGG ACCCTTCCA

49

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CCCGCTAGCA GATCTATGGG GCTGAGCTAT GGAATTTC

39

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

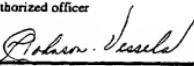
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CGCACCTAGTT GACCCCTCTA TACCATGATC ACTA

34

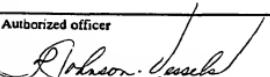
**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**

(PCT Rule 13bis)

<b>A. The indications made below relate to the microorganism referred to in the description on page 37 line 28 and page 38, lines 1-3</b>	
<b>B. IDENTIFICATION OF DEPOSIT</b>	
Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depository institution American Type Culture Collection	
Address of depository institution (including postal code and country) 12301 Parklawn Drive Rockville, Maryland 20852 United States of America	
Date of deposit January 27, 1993	Accession Numbers 75406 and 75405
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
"In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 23(4) EPC)."	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	
<input checked="" type="checkbox"/> This sheet was received with the international application	
Authorized officer 	
For International Bureau use only	
<input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer	

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>39</u> , lines <u>13-16</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b>	
Name of depository institution  American Type Culture Collection	
Address of depository institution ( <i>including postal code and country</i> )  12301 Parklawn Drive Rockville, Maryland 20852 United States of America	
Date of deposit  January 27, 1993	Accession Numbers  75404 and 75403
<b>C. ADDITIONAL INDICATIONS</b> ( <i>leave blank if not applicable</i> ) This information is continued on an additional sheet <input type="checkbox"/>	
<p>"In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 23(4) EPC)."</p>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> ( <i>if the indications are not for all designated States</i> )	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> ( <i>leave blank if not applicable</i> )	
The indications listed below will be submitted to the International Bureau later ( <i>specify the general nature of the indications e.g., "Accession Number of Deposit"</i> )	
<input checked="" type="checkbox"/> For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application	
<input type="checkbox"/> For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer  	Authorized officer

## WE CLAIM:

1. A method for inducing reproducible transient infertility in a mammal which comprises administering to a subject mammal a dose of a zona pellucida protein or fragment thereof, said proteins being selected from 5 the group consisting of mammalian ZPA, mammalian ZPB, and combinations thereof, effective to stimulate production in said mammal of antibodies which recognize ZPA or ZPB protein of said mammal.
2. The method of claim 1, wherein said mammalian ZPA and ZPB are derived from the same mammalian species as the subject 10 mammal.
3. The method of claim 1 wherein said mammalian ZPA and ZPB are derived from a mammalian species other than the subject mammal.
4. The method of claim 1, wherein said mammalian ZPA or 15 ZPB protein is selected from the group consisting of porcine, canine, feline, bovine, cynomolgus monkey, and human ZPA and ZPB.
5. The method of claim 1 wherein said mammalian ZPA and mammalian ZPB are essentially devoid of ZPC.
6. The method of claim 1 wherein said zona pellucida 20 protein is substantially only ZPA.
7. The method of claim 1 wherein said zona pellucida protein is substantially only ZPB.

8. The method of claim 1 wherein said mammalian ZPA and ZPB is recombinant ZPA and ZPB.

9. The method of claim 1 wherein said antibodies have a titer of at least 1:250.

5 10. A method for inducing permanent sterility in a mammal which comprises administering to a subject mammal a dose of a recombinant mammalian ZPC protein or fragment thereof, effective to stimulate production in said mammal of antibodies which recognize the ZPC protein of said mammal.

10 11. The method of claim 10, wherein said mammalian ZPC protein is derived from the same species as the subject mammal.

12. The method of claim 10 wherein said ZPC is derived from a mammalian species other than the subject mammal.

15 13. The method of claim 10, wherein said mammalian ZPC protein is selected from the group consisting of porcine, rabbit, canine, feline, cynomolgus monkey, and bovine ZPC.

14. The method of claim 10 wherein said ZPC protein is essentially devoid of ZPA and ZPB.

20 15. A pharmaceutical composition comprising, an effective contraceptive dose of a recombinant ZPC protein or an immunocontraceptively active fragment thereof.

16. A pharmaceutical composition comprising an effective contraceptive dose of a zona pellucida protein selected from the group consisting of mammalian ZPA and ZPB, and fragments thereof, and pharmaceutically acceptable carriers, diluents and adjuvants.

5 17. The pharmaceutical composition of claim 16 wherein said mammalian ZPA and ZPB are derived from the same mammalian species as the subject mammal.

10 18. The pharmaceutical composition of claim 16, wherein said mammalian ZPA and ZPB are selected from the group consisting of porcine, feline, canine, bovine, cynomolgus monkey, and human ZPA and ZPB.

19. The pharmaceutical composition of claim 16 wherein said mammalian ZPA and ZPB are essentially devoid of ZPC.

15 20. The pharmaceutical composition of claim 16, wherein said mammalian ZPA and ZPB is recombinant ZPA and ZPB.

21. A purified and isolated DNA sequence encoding porcine ZPA, ZPB, ZPC, or immunocontraceptively active fragments thereof, said DNA sequences being essentially as set out in SEQ ID NOS. 1, 3, and 5.

20 22. A purified and isolated DNA sequence encoding rabbit ZPC or an immunocontraceptively active fragment thereof, said DNA sequences being essentially as set out in SEQ ID NO. 7.

23. A purified and isolated DNA sequence encoding canine ZPA or ZPC, or immunocontraceptively active fragments thereof, said DNA sequences being essentially as set out in SEQ ID NOS. 9 and 11.

24. A purified and isolated DNA sequence encoding feline ZPA, ZPB, or ZPC, or immunocontraceptively active fragments thereof, said DNA sequences being essentially as set out in SEQ ID NOS. 13, 15, and 17.

25. A purified and isolated DNA sequence encoding bovine ZPA, ZPB, or ZPC, or immunocontraceptively active fragments thereof, said DNA sequences being essentially as set out in SEQ ID NOS. 19, 21, and 23.

26. A purified and isolated DNA encoding human ZPA or immunocontraceptively active fragments thereof, comprising DNA present in the human DNA inserts in lambda phage clones A1 (ATCC No. 75404) and A4 (ATCC No. 75403).

27. A purified and isolated DNA encoding human ZPA or an immunocontraceptively active fragment thereof, said sequence being essentially as set out as SEQ ID NO. 42.

28. A purified isolated DNA encoding human ZPB or immunocontraceptively active fragments thereof, comprising human DNA present in the DNA inserts in lambda phage clones 1-1 (ATCC No. 75406) and 4-9 (ATCC No. 75405).

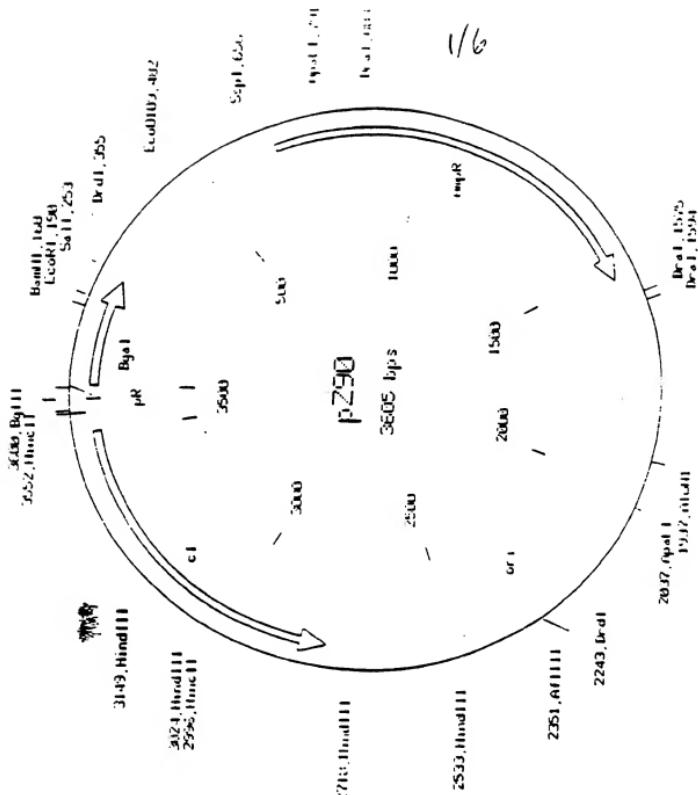
29. A purified and isolated DNA encoding human ZPB or an immunocontraceptively active fragments thereof, said sequence being essentially as set out in SEQ ID NO. 40.

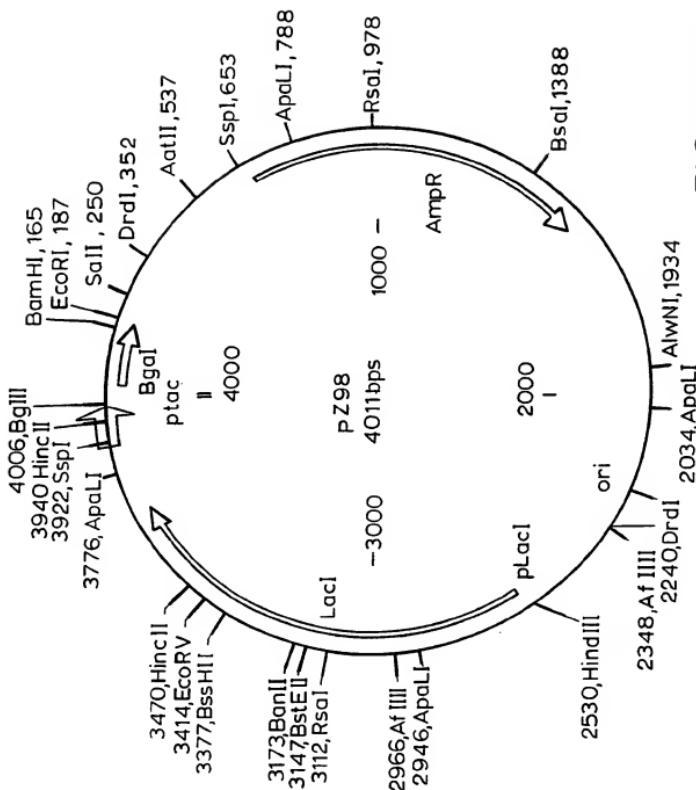
30. A vector containing the DNA sequence of claim 21.
31. A vector containing the DNA sequence of claim 22.
32. A vector containing the DNA sequence of claim 23.
33. A vector containing the DNA sequence of claim 24.
- 5 34. A vector containing the DNA sequence of claim 25.
35. A vector containing the DNA sequence of claim 26.
36. A vector containing the DNA sequence of claim 27.
37. A vector containing the DNA sequence of claim 28.
38. A vector containing the DNA sequence of claim 29.
- 10 39. A prokaryotic or eucaryotic host cell stably transformed or transfected with a vector according to claims 30, 31, 32, 33, 34, 35, 36, 37, or 38.
- 15 40. A polypeptide product of the expression in a prokaryotic or eucaryotic host cell of a DNA sequence according to claims 21, 22, 23, 24, 25, 26, 27, 28 or 29.
41. A process for the production of a recombinant mammalian zona pellucida protein or fragment thereof, said process comprising:

growing, under suitable nutrient conditions, procaryotic or eucaryotic host cells transformed or transfected with a DNA vector according to claims 30, 31, 32, 33, 34, 35, 36, or 37 and isolating desired polypeptide products of the expression of DNA sequences in said vector.

5           42. A method for inducing reproducible transient infertility in a mammal, the method comprising, administering to a subject mammal a contraceptively effective dose of an antibody directed to a zona pellucida protein, said antibody selected from the group consisting of anti-ZPA antibodies and anti-ZPB antibodies.

10           43. A method for inducing permanent sterility in a mammal, the method comprising administering to a subject mammal a contraceptively effective dose of an antibody directed to ZPC.





**FIGURE 2**

**RECTIFIED SHEET (RULE 91)**

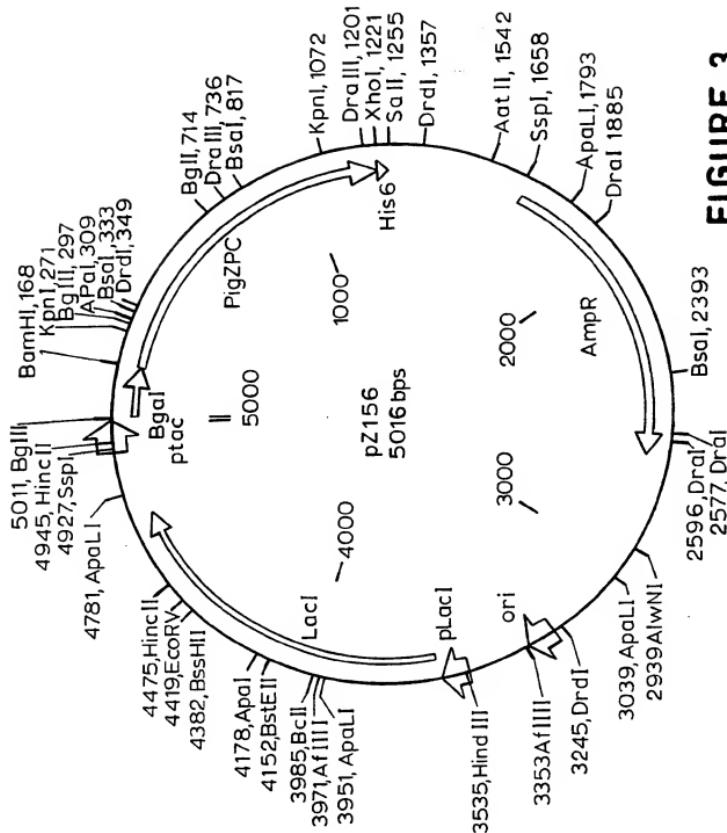
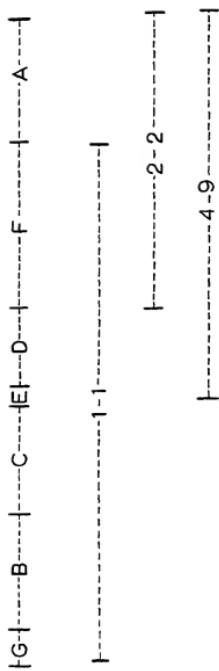
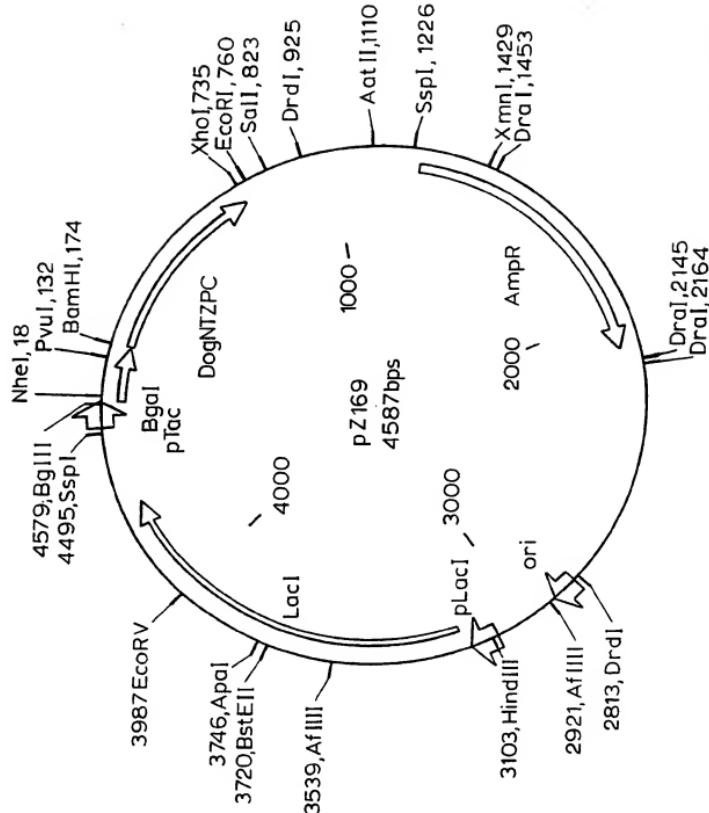
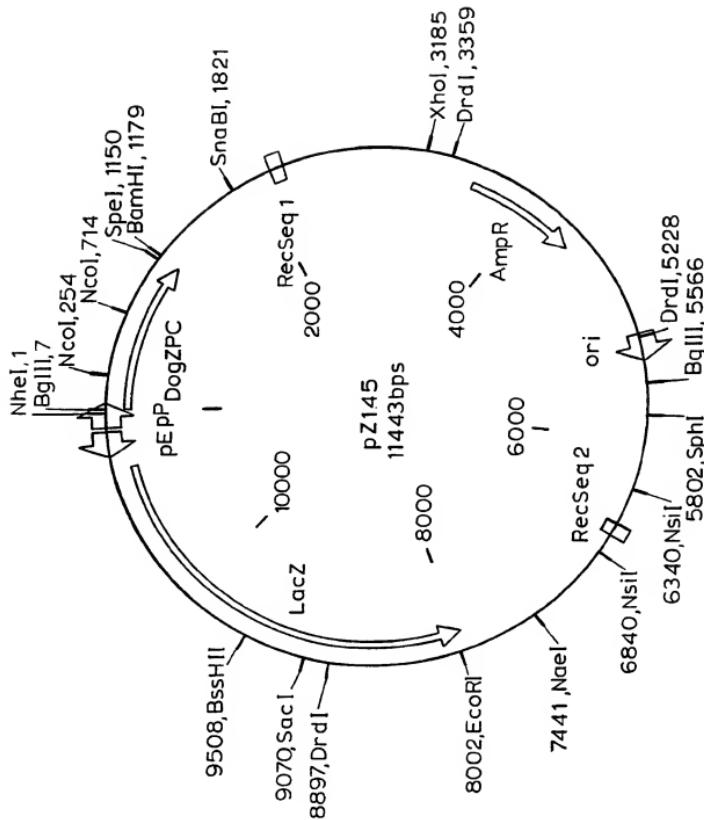
**FIGURE 3**

FIGURE. 4



RECTIFIED SHEET (RULE 91)

**FIGURE 5**

**FIGURE 6**

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/10851

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC(S) :A61K 37/02, 39/00, 39/35; C07K 13/00; C12N 5/10, 15/12; C12P 21/00 US CL :424/85.8, 88; 435/69.1, 69.3, 320.1; 536/23.1, 23.5 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)  U.S. : 424/85.8, 88; 435/69.1, 69.3, 320.1; 536/23.1, 23.5		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  APS, DIALOG, BIOSIS, EMBASE, MEDLINE, WPI search terms: harris, zona pellucida, ZP3, ZPA,ZPB, ZPC, contraception		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 4,996,297 (Dunbar) 26 February 1991, see entire document.	1-43
Y	WO 90/15624 (Dean) 27 December 1990, see entire document.	1-43
Y	WO 92/03548 (Van Duin) 05 March 1992, see entire document.	1-43
Y	Proc. Natl. Acad. Sci., Volume 87, issued August 1990, M.E. Chamberlin et al., "Human Homolog of the Mouse Sperm Receptor", pages 6014-6018, see entire document.	1-43
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
<ul style="list-style-type: none"> <li>* Special categories of cited documents:</li> <li>"A" documents defining the general state of the art which is not considered to be part of particular relevance</li> <li>"E" earlier documents published on or after the international filing date</li> <li>"L" documents which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</li> <li>"O" documents referring to an oral disclosure, use, exhibition or other means</li> <li>"P" documents published prior to the international filing date but later than the priority date claimed</li> </ul>		<ul style="list-style-type: none"> <li>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</li> <li>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</li> <li>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</li> <li>"&amp;" document member of the same patent family</li> </ul>
Date of the actual completion of the international search  31 January 1994		Date of mailing of the international search report  MAR 11 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE		Authorized officer  PHILLIP GAMBLE Telephone No. (703) 305-0196

## INTERNATIONAL SEARCH REPORT

I: national application No. PCT/US93/10851
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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Developmental Biology, Volume 127, issued October 1988, M.J. Ringuette et al., "Molecular Analysis of cDNA Coding for ZP3, a Sperm Binding Protein of the Mouse Zona Pellucida", page 287-295, see entire document.	1-43
Y	Biology of Reproduction, Volume 44, issued April 1992, J.A. Keenan et al., "Endocrine Response in Rabbits Immunized with Native Versus Deglycosylated Porcine Zona Pellucida Antigens, page 150-156, see entire document.	1-43
Y	Biology of Reproduction, Volume 41, issued December 1989, A.G. Sacco et al., "Porcine Zona Pellucida: Association of Sperm Receptor Activity with the alpha-Glycoprotein Component of the Mr=55,000 Family", pages 523-532, see entire document.	1-43
Y	J. Biol. Chem., Volume 262, issued 15 January 1987, E.C. Yurewicz et al., "Structural Characterization of the Mr=55,000 Antigen (ZP3) of Porcine Oocyte Zona Pellucida", pages 564-571, see entire document.	1-43

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/10851BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING  
This ISA found multiple inventions as follows:

- I. Claims 1-9, 16-20, 40 and 42 drawn to a method of inducing transient infertility and pharmaceutical compositions comprising ZPA or ZPB proteins, classified in Class 424, subclass 88 and 85.8.
- II. Claims 10-15, 40 and 43 drawn to a method of inducing permanent sterility and pharmaceutical compositions with ZPC proteins, classified in Class 424, subclass 88 and 85.8.
- III. Claims 21-39 and 41, drawn to DNA and expression vectors for zona pellucida proteins and a process of producing recombinant proteins, classified in Class 435, subclasses 69.1 and 69.3, 320.1 and Class 536, subclasses 22.1 and 23.5.

The inventions listed as Groups I/II/III do not meet the requirements for Unity of Invention for the following reasons:

Group I is drawn to a first product and a first method of use, Group II is drawn to second product and a second method of use; and Group III is drawn to a third product. PCT Rule 13 does not provide for multiple products or methods within a single application. These inventions require different ingredients and process steps to accomplish the use of ZPA-, ZPB-, ZPC-specific proteins and ZPA-, ZPB-, ZPC-specific antibodies. Proteins (pharmaceutical compositions) and DNA (and its vectors) are distinct because their structures and modes of action are different. Furthermore, this application contains claims directed to the following patentably distinct species of the claimed inventions I, II and III: wherein the zona pellucida protein specificity is (a) ZPA, (b) ZPB or (c) ZPC. These species are distinct because their structures and modes of action are different; the substitution of one for another would not lead to the same effects.

